

21 December 2012 | S16

Science

BREAKTHROUGH
of the YEAR

The **HIGGS
BOSON**

AAAS

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Breakthrough of the Year

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Photos: Maximilien Brice and Claudia Marcelloni/CERN

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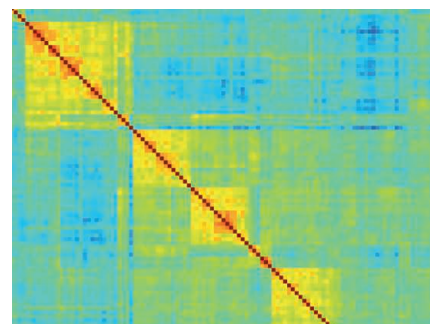
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www.sciencexpres.org
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C. Ma et al.
10.1126/science.1230473

Comparative Analysis of Bat Genomes Provides Insight into the Evolution of Flight and Immunity

G. Zhang et al.
10.1126/science.1230835

An Update of Wallace's Zoogeographic Regions of the World

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Cyclic GMP-AMP Is an Endogenous Second Messenger in Innate Immune Signaling by Cytosolic DNA

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Cyclic GMP-AMP Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway

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Boson Sampling on a Photonic Chip

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Photonic Boson Sampling in a Tunable Circuit

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Comment: S. Roorda and L. J. Lewis
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SCIENCE NOW

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Highlights From Our Daily News Coverage

Cloud Forest Trees Drink From the Fog

Plants take up a surprising amount of moisture from the air.
http://scim.ag/Moisture_Air

Revved-Up Protein Fights Aging

Boosting a protein that keeps chromosomes in order protects mice from cancer and increases life span.
http://scim.ag/Protein_Aging

Pee Marks the Spot

A single protein in male mouse urine makes females return to old haunts.
<http://scim.ag/Single-Protein>

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www.sciencesignaling.org
The Signal Transduction Knowledge Environment
18 December issue: <http://scim.ag/ss121812>

RESEARCH ARTICLE: IGFBP7 Binds to the IGF-1 Receptor and Blocks Its Activation by Insulin-like Growth Factors

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RESEARCH ARTICLE: c-FLIP Maintains Tissue Homeostasis by Preventing Apoptosis and Programmed Necrosis

X. Piao et al.
The anti-apoptotic protein c-FLIP blocks multiple cell death pathways in mice.

RESEARCH ARTICLE: A CC-SAM, for Coiled-Coil–Sterile α Motif, Domain Targets the Scaffold KSR1 to Specific Sites in the Plasma Membrane

D. Koveal et al.
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REVIEW: Extracellular Phosphorylation and Phosphorylated Proteins—Not Just Curiosities But Physiologically Important

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SCIENCE TRANSLATIONAL MEDICINE

www.sciencetranslationalmedicine.org
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COMMENTARY: To Share or Not To Share—That Is Not the Question

L. Ohno-Machado
Sharing clinical and biomedical data could accelerate translational research.

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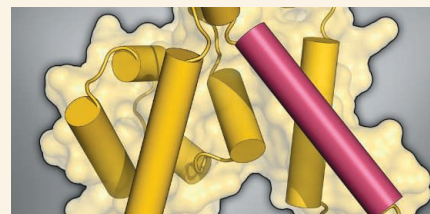
G. D. Brown et al.
Lack of rapid diagnostic tests, drugs, and vaccines impedes treatment and prevention of invasive fungal infections.

RESEARCH ARTICLE: Genetic Correction of Human Induced Pluripotent Stem Cells from Patients with Spinal Muscular Atrophy

S. Corti et al.
Induced pluripotent stem cell–derived motor neurons from spinal muscular atrophy patients show phenotype rescue after genetic correction.

RESEARCH ARTICLE: Long-Term Follow-Up After Gene Therapy for Canavan Disease

P. Leone et al.
Gene therapy for Canavan disease results in a decrease in pathologically elevated *N*-acetyl-aspartate concentrations in the brain and long-term clinical stabilization.



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Membrane-binding domain in KSR1.

RESEARCH ARTICLE: Multimodal Actions of Neural Stem Cells in a Mouse Model of ALS—A Meta-Analysis

Y. D. Teng et al.
A meta-analysis reports the beneficial effects of transplanting mouse or human neural stem cells into the spinal cord of the SOD1^{G93A} mouse, a model of ALS.

SCIENCE CAREERS

www.sciencereers.org/career_magazine
Free Career Resources for Scientists
<http://scim.ag/SciCareers21December2012>

Breakthrough of the Year: Seekers of the Higgs Boson

E. Pain
Science Careers talks to three young investigators who contributed to this year's monumental discovery.
>> Breakthrough of the Year section p. 1524

Science Careers's Person of the Year:

Paula Stephan
B. L. Benderly
The labor economist has worked for years behind the scenes, but this year she went public.

SCIENCE PODCAST

www.sciencemag.org/multimedia/podcast
Free Weekly Show for 21 December 2012
A special year-in-review show featuring the Breakthrough of the Year and Runners-Up and science news highlights from 2012.

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The Meteor That Fell to Earth >>

In April 2012, a meteor was witnessed over the Sierra Nevada Mountains in California. **Jenniskens *et al.*** (p. 1583) used a combination of photographic and video images of the fireball coupled with Doppler weather radar images to facilitate the rapid recovery of meteorite fragments. A comprehensive analysis of some of these fragments shows that the Sutter's Mill meteorite represents a new type of carbonaceous chondrite, a rare and primitive class of meteorites that contain clues to the origin and evolution of primitive materials in the solar system. The unexpected and complex nature of the fragments suggests that the surfaces of C-class asteroids, the presumed parent bodies of carbonaceous chondrites, are more complex than previously assumed.



Whence Species Variation?

Vertebrates have widely varying phenotypes that are at odds with their much more limited protein-coding genotypes and conserved messenger RNA expression patterns. Genes with multiple exons and introns can undergo alternative splicing, potentially resulting in multiple protein isoforms (see the Perspective by **Papasaïkas and Valcárcel**). **Barbosa-Morais *et al.*** (p. 1587) and **Merkin *et al.*** (p. 1593) analyzed alternative splicing across the genomes of a variety of vertebrates, including human, primates, rodents, opossum, platypus, chicken, lizard, and frog. The findings suggest that the evolution of alternative splicing has for the most part been very rapid and that alternative splicing patterns of most organs more strongly reflect the identity of the species rather than the organ type. Species-classifying alternative splicing can affect key regulators, often in disordered regions of proteins that may influence protein-protein interactions, or in regions involved in protein phosphorylation.

Enhancing Heart Function

The epicardium, a protective layer of tissue surrounding the mammalian heart, plays a critical role during embryogenesis because it supplies growth factors and multipotent progenitor cells essential for heart development. In adults, the epicardium is dormant but it becomes reactivated when the heart is injured, a response that leads to re-expression of developmental genes. Studying mouse models, **Huang *et al.*** (p. 1599, published online 15 November; see the Perspective by **Rosenzweig**) found that the C/EBP transcription

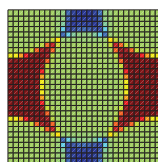
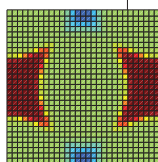
factors activated the epicardium during development and injury. Blockade of C/EBP signaling in the epicardium of injured (ischemic) hearts reduced inflammation and improved heart function, a finding that could ultimately lead to new strategies for the repair of heart damage.

Symmetry Semantics

Topological insulators (TIs) are characterized by boundary states that are protected by time-reversal symmetry. A systematic study of this, and other symmetry-protected states, is possible in noninteracting systems, but complications arise when interactions are present. **Chen *et al.*** (p. 1604; see the Perspective by **Qi**) used group cohomology theory to predict symmetry-protected phases of interacting bosons. The analysis enabled the generalization of a known result in one dimension by using a path-integral formulation and suggests the existence of three counterparts of TIs in three dimensions, and one in two dimensions, as well as phases protected by other symmetries. The formalism is applicable to any symmetry group and dimension and is valid for interactions of arbitrary strength.

Getting Rid of Negativity

Problems in condensed matter physics involving strong interactions are notoriously hard to tackle analytically, and physicists often resort to numerical methods such as quantum Monte Carlo (QMC).



However, in the most interesting cases, this method becomes computationally intractable because partition sums for fermionic systems

generally involve integration over an oscillatory function with both negative and positive values. **Berg *et al.*** (p. 1606) find a way around this problem for a two-dimensional metal in the vicinity of an antiferromagnetic quantum phase transition, which is of relevance to electron-doped cuprates, iron-based superconductors, and heavy fermion compounds. Their lattice theory results in a positive integrand for the partition sums; thus, avoiding the sign problem; and yields the expected competition between antiferromagnetic order and unconventional superconductivity near the critical point.

Inducing a Quiet Space

The interaction between light and matter forms the foundation of many applications in communication and sensing, as well as provides insights into fundamental quantum-level processes. Optical coupling of a mechanical system can be used to study these processes. However, because the mechanical oscillator is unavoidably coupled to its environment, thermal noise can spoil the sensitivity of the optomechanical coupling. **Dong *et al.*** (p. 1609, published online 15 November) exploit the ability to form a mechanical "dark state" that can effectively isolate the mechanical system from thermal-induced noise. The formation of such a noise-free zone may provide a simpler route to probe quantum optomechanical systems that circumvents the need to cool the oscillator to its quantum limit where all thermal motion is frozen out.

Earning a High Grade

Most of the world's copper and molybdenum come from porphyry-type ore deposits in Earth's

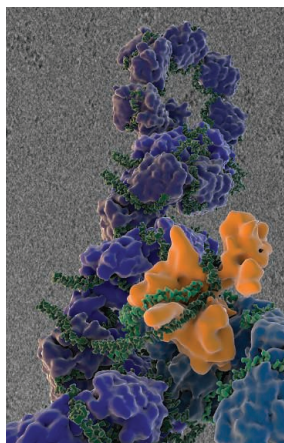
crust. The metals are deposited either as veins of concentrated metals in fractured rock or in a confined shell, associated with the edges of magma chamber plumes. But it remains unclear why a front of sharp temperature-pressure gradients, which allows the accumulation of high-grade metal deposits, remains stable. **Weis *et al.*** (p. 1613, published online 15 November; see the Perspective by **Ingebritsen**) constructed a hydrothermal model of a porphyry-type system given a supply of magmatic fluids with high metal concentrations. The thermodynamic properties of injected volatile fluids and dynamic variations in host rock permeability, driven by injection-induced fracturing, controlled the stability and evolution of the fronts and allowed for the formation of an extensive network of ore veins from within the magmatic chamber.

A Grand Old Canyon

In the southwestern United States, the Grand Canyon is a striking example of the power of erosion over time. Over millions of years, flowing river water carved out the canyon that today measures over 1.6 km deep and 29 km long. Most models posit that the majority of the canyon formed 5 to 6 million years ago. Using thermochronometry, **Flowers and Farley** (p. 1616, published online 29 November) examined the temperature-dependent diffusion of helium within mineral grains representative of the canyon basement, which cools as erosion brings crustal rocks near the surface. After validating the approach across the younger eastern canyon, the model suggests that the western canyon experienced an ancient cooling event induced by erosional processes, such that the canyon likely reached near modern depths by 70 million years ago—nearly 60 million years earlier than generally believed.

Influenza Revealed

Influenza virus, a single-stranded RNA virus, is responsible for substantial morbidity and mortality worldwide. The influenza ribonucleoprotein (RNP) complex, which carries out viral replication and transcription, is central to the virus life-cycle and to viral host adaptation (see the Perspective by **Tao and Zheng**). Structural characterization of the viral RNP has been challenging, but **Moeller *et al.*** (p. 1631, published online 22 November) and **Arranz *et al.*** (p. 1634, published online 22 November) now report the structure and assembly of this complex, using cryo-electron microscopy and negative-stain electron microscopy. The structures reveal how the viral polymerase, RNA genome, and nucleoprotein interact in the RNP providing insight into mechanisms for influenza genome replication and transcription.



Autism Genes, Again and Again

Despite recent advances in sequencing technologies and their lowered costs—effective, highly sensitive, and specific sequencing of multiple genes of interest from large cohorts remains expensive. **O’Roak *et al.*** (p. 1619; published online 15 November) modified molecular inversion probe methods for target-specific capture and sequencing to resequence candidate genes in thousands of patients. The technique was applied to 44 candidate genes to identify *de novo* mutations in a large cohort of individuals with and without autism spectrum disorder. The analysis revealed several *de novo* mutations in genes that together contribute to 1% of sporadic autism spectrum disorders, supporting the notion that multiple genes underlie autism-spectrum disorders.

Single-Cell Sequencing

With the rapid progress in sequencing technologies, single-cell sequencing is now possible, promising insight into how cell-to-cell heterogeneity affects biological behavior. Achieving adequate genome coverage remains a challenge because single-cell sequencing relies on genome amplification that is prone to sequence bias. **Zong *et al.*** (p. 1622) report a new amplification method: multiple annealing and looping-based amplification cycles that allowed 93% genome coverage for a human cell. This coverage facilitated accurate detection of point mutations and copy number variations. **Lu *et al.*** (p. 1627) used the method to sequence 99 sperm cells from a single individual. Mapping the meiotic crossovers revealed a nonrandom distribution with a reduced recombination rate near transcription start sites.

CREDIT: ARNE MOELLER



Bruce Alberts is Editor-in-Chief of *Science*.

The Breakthroughs of 2012

WE AT *SCIENCE* HAVE ONCE AGAIN HAD THE CHALLENGE OF CHOOSING 10 SCIENTIFIC accomplishments to highlight in this final issue of the year. Our 10 choices reveal that 2012 has been a remarkable one for the physics of particles (the Higgs boson, neutrinos, and Majorana “quasiparticles”), as well as for biological discovery (the production of eggs from stem cells, the derivation of some modern human genes from Denisovan ancestors, and the greatly enhanced cataloging of human genetic regulation). Three technological breakthroughs also appear on our list: functional brain/machine interfaces, TALENS as a tool for genetic engineering, and the x-ray laser determination of a protein structure. And finally, there is one feat of physics and engineering virtuosity: the Curiosity rover’s remarkably precise, gentle, “sky crane” landing on Mars.

The top Breakthrough of the Year—the discovery of the Higgs boson—was an unusually easy choice, representing both a triumph of the human intellect and the culmination of decades of work by many thousands of physicists and engineers. The two teams at the CERN Large Hadron Collider that detected this elusive particle have in this issue provided a more widely accessible version of their detailed findings published earlier this year in *Physics Letters B*. These articles are accompanied by a historical summary and overview of this seminal discovery (p. 1524).

The physicist Pierre Hohenberg suggests that it would be useful to distinguish “between the activity of scientists and the product of that activity by denoting the former as (lower-case) science and the latter as (upper-case) Science.”* In this view, “Science emerges from science” as “collective, public knowledge...universal and free of contradiction” only after being repeatedly tested by independent scientific investigations. The “standard model” of particle physics, which explains how interactions among the most basic subatomic particles lead to observed matter and forces, is now confirmed by the Higgs boson discovery. It certainly represents big “S”cience, having been challenged and refined over the course of the past 40 years through the efforts of tens of thousands of physicists. And yet, a great deal of mystery still remains at a fundamental level about how the universe behaves.†

An example of outstanding “s”cience forms the basis for this year’s breakthrough on Majorana fermions, the first report of a “particle” that is its own antiparticle, formed from the collective motion of many interacting electrons.‡ But further work will be needed, both by the discoverers themselves and by other scientists, to be certain that there is no other way of explaining the results. Only then will this new science become Science.

The success of Science over the past few centuries has enabled humans to reach a remarkable understanding of the natural world that makes our lives much more stable and predictable, just as it enabled scientists and engineers to deliver a 3.3-metric-ton cargo—the Mars rover and its landing craft—onto the surface of a distant planet after traveling for 563 million kilometers. Thus, it is through Science that we know that cigarette smoking over several decades has a high probability of inducing lung cancer; and that, over an even longer time span, human-induced greenhouse gas emissions will endanger life for our descendants on Earth.

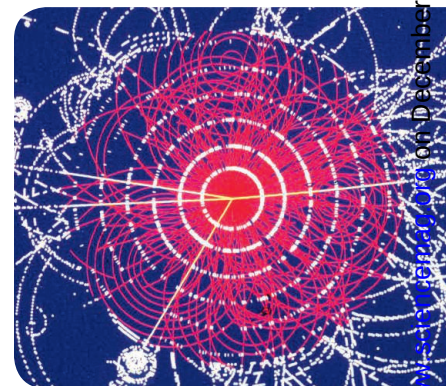
Individuals, communities, and nations must all make wise long-range decisions based on what scientists do and do not know. Everyone therefore needs to understand the difference between science and Science and the critical, evidence-based process of getting from one to the other. It is deeply discouraging that in the United States, many political leaders feel comfortable denying the Science of climate change. The acceptability of this stance represents a general failure of science education and communication. It is but one grave example that should spur scientists to focus much more effort on the critical task of ensuring that students, and the general public, understand exactly how Science is derived from science.

— Bruce Alberts

10.1126/science.1234108

*http://physics.nyu.edu/~pch2/What_is_Science-December_2010.pdf.

†www.sciencemag.org/site/special/astro2012/index.xhtml. ‡V. Mourik *et al.*, *Science* **336**, 1003 (2012).





PLANT SCIENCE

A Productively Repellant Aura

The domesticated tomato has wild relatives which, although they may lack large juicy fruits, retain useful defenses against insect infestation. The reduced density of trichomes and absence of specific biosynthetic pathways leave the domesticated tomato more susceptible to insect infestation, which can cause devastating crop losses. Bleeker *et al.* have now identified key enzymes in the biosynthetic pathway for 7-epizingiberene, a terpene derivative exuded from leaf trichomes of the wild, but not domesticated, tomato that repels whiteflies and spider mites. Cross-breeding experiments showed that plants that expressed some 7-epizingiberene, even if it was less than the expression seen in wild tomato plants, were resistant to whitefly pests. Expression of 7-epizingiberene synthase (ShZIS) and *cis*-prenyltransferase (zFPS), both from wild tomato, in trichomes of domesticated tomato drove production of 7-epizingiberene. The consequent reduction of whitefly and spider mite infestations suggests routes to protect domesticated tomato crops. — PJH

Proc. Natl. Acad. Sci. U.S.A. **109**, 20124 (2012).

CELL BIOLOGY

Barrier Maintenance

The central nervous system (CNS) and retina are privileged areas of blood vessel growth and function. For example, the specialized vasculature restricts the diffusion of toxic molecules and the entry of pathogens into these regions. Frizzled4, a protein expressed at the surface of vascular endothelial cells (ECs), and its activating ligand Norrin are important for the development of blood vessel networks. Wang *et al.* now report that in the mouse, Norrin-Frizzled4 signaling is required for early vascular development in the retina and also later in the CNS to maintain vascular integrity. In genetically engineered mice lacking either Norrin or Frizzled4, retinal blood vessel growth was slow, formed irregular, criss-crossed patterns, and failed to create a trilayer architecture. The authors could also visualize leakage of these blood vessels in regions of the brain, spinal cord, olfactory bulb, and retina, and show that Norrin-Frizzled4 signaling is needed for the expression of cell junction proteins. In mice engineered to lack Frizzled4 in only a few percent of the EC population, general vascular organization appeared normal, but was leaky only in regions of blood vessels where Frizzled4 was absent. The loss of either the blood-brain or blood-retina barrier occurs in many disorders, including stroke, diabetes, infections, and eye disease. The Norrin-Frizzled4 signaling pathway may thus be a new therapeutic target. — LC

Cell **151**, 1332 (2012).

GEOLOGY

A Map of Earth's Rocks

The distribution of rocks on Earth's continents reflects their geologic history spanning deposition, formation, deformation, metamorphism, uplift, and erosion. It also has important implications for engineering and resources, and the rocks exposed in areas of erosion influence global ocean chemistry and the composition of dust in the atmosphere. Hartmann and Moosdorf have compiled a global map of rock types exposed on Earth's continents using a variety of individual geologic maps and literature resources. Their high-resolu-



tion global map shows that most of the exposed rocks are sedimentary in origin, and most of these and about 20% of the total are carbonate rocks. Metamorphic rocks make up about 13%, as do igneous rocks, distributed roughly equally between volcanic and plutonic rocks. About 10% of Earth's continents are covered in ice or water. Rock types are distributed unequally over the continents;

Africa is covered by extensive metamorphic rocks, whereas siliciclastic rocks (e.g. sandstones, shales, and conglomerates) dominate in North America and northern Eurasia. — BH

Geochem. Geophys. Geosys. **13**, Q12004 (2012).

EDUCATION

Summing Up Math Standards

Are the Common Core State Standards for Mathematics (CCSSM), which are about to be implemented in the United States, high-quality standards that are internationally competitive? Using data from standards of the highest-achieving nations on the 1995 Trends in International Mathematics and Science Study (TIMSS) and all 50 state standards in place in 2008, Schmidt and Houang analyzed the focus, coherence, and rigor of the content defined by the CCSSM in an effort to predict their impact on student achievement.

Comparison of CCSSM with the international standards revealed an almost 90% degree of consistency, suggesting that the CCSSM are focused, rigorous, and worthy of being world-class standards. When applied to state standards, the same analysis showed a wide variation, suggesting that implementation of the CCSSM will be easier for some states than others. Implementing

the CCSSMM will be worth the effort, however, as further analysis revealed that states with existing standards most similar to the CCSSM had higher National Assessment of Educational Progress scores. Although the analyses from this study are an indication of correlation and not of causality, they do suggest that the CCSSM, once implemented, will improve the mathematics achievement of U.S. students. — MM

Educational Researcher 41, 294 (2012).

PHYSICS

A Single-Atom Lasso

Optical tweezing is a powerful technique for trapping and manipulating particles and can be applied to a broad range of size scales—from single cells and viruses to glass beads several micrometers in diameter. Tweezing can also be used to trap single atoms. However, the interaction between photons in the trapping light beam and the atom generally results in atom jitter. This motion of the atom tends to prevent the atoms from being cooled to the lowest temperatures, where interesting quantum effects can then be probed. Kaufman *et al.* used an optical trapping beam and a suite of laser pulses to lasso a single rubidium atom and exploited Raman transitions of the atom to cool it to the quantum ground state. Once trapped and cooled to remove all vibrations from the atom, they also show that they could coherently manipulate its quantum spin and motion. The generality and flexibility of the optical tweezing approach may allow more complex systems comprising arrays of overlapping and interacting trapped atoms or molecules to be designed to form quantum simulators (i.e., well-controlled, engineered quantum systems that can be used to model other less-well-understood condensed-matter systems). — ISO

Phys. Rev. X 2, 041014 (2012).

GENETICS

Crossovers and Cancer

Many cancer-related genes involve variants of genes involved in DNA repair, which often also play a role in chromosomal recombination. Hussin *et al.* used exome sequencing to analyze a family with two siblings that have childhood B cell precursor acute lymphoblastic leukemia (B-ALL). They found that the mother carried a rare variant of PRDM9, a protein that regulates recombination hotspot usage in humans. Examination of the recombination profiles between the parents and offspring revealed an unusual maternal recombination profile in the two B-ALL-affected siblings. The authors then sequenced the exomes of a cohort

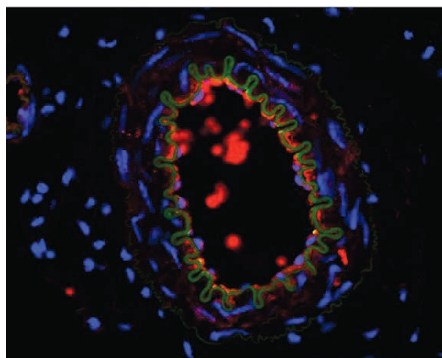
of 44 parents whose children are affected by B-ALL and identified an excess of rare alleles relative to control populations. These findings were confirmed in an independent cohort of B-ALL-affected children and infants. Analysis of the motifs bound by the PRDM9 variants revealed that they were more likely to be found in segmental duplications within the genome in genes associated with ALL. On the basis of their investigations, the authors propose that PRDM9-mediated effects on meiotic recombination may contribute to the development of childhood leukemogenesis. — LMZ

Genome Res. 10.1101/gr.144188.112 (2012).

CELL BIOLOGY

Feeling the Stretch

Certain cells in the body, particularly those of the vasculature, need to respond appropriately when they are stretched. One way they do this is through the protein zyxin. In stretched cells, zyxin is released from focal adhesions (where cells are attached to their surroundings) and



goes to the nucleus, where it functions as a transcription factor to regulate gene expression. Suresh Babu *et al.* reveal a cellular signaling mechanism by which mechanical stretching is coupled to zyxin release. Using genetically modified mice, they found that zyxin release in response to stretching requires TRPC3 (transient receptor potential channel 3) protein, an ion channel from a family in which some members are regulated by mechanical stimuli. They showed that the channel activation appeared to cause release of endothelin-1 from mammalian endothelial cells, which then acted through its receptor to cause release of atrial natriuretic peptide, whose receptor is a guanylyl cyclase. Production of cyclic GMP (adenosine 3'-5' monophosphate) would then activate protein kinase G, which phosphorylates zyxin, an event that appears to be necessary for its release from the focal adhesions. — LBR

Sci. Signal. 5, ra91 (2012).

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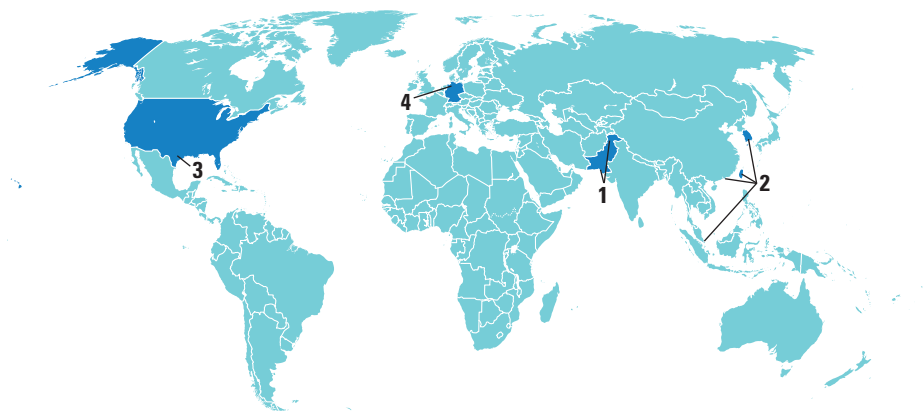
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Karachi and Peshawar, Pakistan 1

Six Polio Campaign Workers Killed

Six vaccination campaign workers were killed and others injured in attacks in Pakistan on 17 and 18 December, prompting the country's health officials to suspend the country's 3-day mass vaccination campaign in greater Karachi. Vaccinations are going ahead in Peshawar, a city in the northwestern part of the country. Three appar-



ently coordinated attacks in poor Karachi neighborhoods claimed five victims. One volunteer was killed in Peshawar. Taliban insurgents have repeatedly threatened campaign workers, but when *Science* went to press, no one had claimed responsibility for the attacks.

Pakistan is one of the world's three remaining polio hotspots (*Science*, 3 August, p. 517). The killings occurred as the country is making significant progress against the disease; there have been only 56 cases so far this year, down from 173 this time in 2011. The worst reaction to these "horrible, awful" events would be to let this opportunity be squandered, says Bruce Aylward of the World Health Organization, who leads the global effort to eradicate polio.

Singapore, Hong Kong, Taiwan, and South Korea 2

East Asian Students Still Excel In Math, Science

The latest results from the Trends in International Mathematics and Science Study (TIMSS) released last week show that fourth- and eighth-grade students from Singapore, Hong Kong, Taiwan, and South Korea have retained—and in some cases widened—their lead over the rest of the 63 countries that took the tests in 2011. "Revolutionary results require revolutionary changes," says Michael Martin, co-director of the International Study Center at Boston College that administers the quadrennial TIMSS as well as PIRLS, a similar test of reading and literacy skills. Those changes are more likely to occur, he says, in countries that have a centralized education system and can move quickly to embrace the latest thinking on how to improve schooling.

U.S. students placed 11th in fourth-grade math, ninth in eighth-grade math, seventh in fourth-grade science, and 10th in eighth-grade science. Fourth-graders showed significant gains in math, whereas U.S. scores in the other three categories remained flat. <http://scim.ag/TIMSS2011>

Austin 3

More Changes at Texas Cancer Agency

William Gimson, the executive director of the troubled \$3 billion Cancer Prevention and Research Institute of Texas (CPRIT), stepped down last week. The agency also announced a new chief scientific officer to replace Alfred Gilman, the Nobel laureate who quit in protest over the agency's peer review procedures. Gimson explained in a letter to CPRIT's

board that after 8 months of controversy, he has "been placed in a situation where I feel I can [no] longer be effective." His resignation follows the departure of CPRIT Chief Commercialization Officer Jerald Cobbs in November. Last week, the Travis County district attorney launched a criminal investigation into CPRIT's funding of several commercialization awards. CPRIT's new chief scientist is Margaret Kripke, a cancer immunologist and former executive vice president at the University of Texas MD Anderson Cancer Center in Houston. She told reporters that her first task will be to rebuild CPRIT's "really terrific" peer review system, which lost many reviewers when Gilman left in October. <http://scim.ag/CPRITchanges>



Bremen, Germany 4

German Court OKs Macaque Research

A long-running legal fight over animal research in Germany ended last week when a court ruled that University of Bremen neuroscientist Andreas Kreiter could continue his research on macaques. In 2008, Bremen authorities refused to renew Kreiter's license for animal research, claiming that the knowledge gained from the experiments did not justify the suffering endured by the macaques, which have electrodes implanted into their brains. Kreiter and the university sued, and in 2009 a court ruled in their favor.

The city-state of Bremen appealed—but the latest ruling, made on 11 December, found that the authorities were not justified in denying Kreiter his license, and ruled out further appeals. Research organizations welcomed the ruling as a fundamental decision in favor of the "freedom of research" clause in German law. (Kreiter had been allowed to continue his research during the court battle.) Activists opposed to the research have vowed to continue their fight through political means.

THEY SAID IT

"It's like judging the bottles in a wine contest by the labels only without tasting their content."

—Political economist **Alberto Baccini** of the University of Siena in Italy, speaking about a major government effort to evaluate Italian scientists and research institutions.
<http://scim.ag/Italyex>

NEWSMAKERS

Three Q's

What is time and how would you explain it to an 11-year-old? That's the second Flame Challenge that actor **Alan Alda** and the Center for Communicating Science at Stony Brook University in New York have posed to scientists. Answers are due 1 March and will be judged by thousands of children. (Read the full interview at <http://scim.ag/AldaFlame>.)



Alda

Q: Does the Flame Challenge reflect the center's mission?

Yes. Can I explain something to an 11-year-old with the 11-year-old judging how well I do? That's fun for everybody. But the scientist is really being challenged to see if he or she can remember what it's like not to know what they now know so intimately. To break it all down and yet not take forever getting through it, that's a real challenge.

Q: Isn't this year's question particularly difficult?

It sure is! The first question—what is a flame?—came from me and my memories of when I was an 11-year-old. But this year's question comes from current 11-year-olds. And they're asking a much deeper question.

Q: So the judgment is done by vote?

The top vote wins. The children are very careful about dismissing the answers that they think are too short and not informative enough. They don't mind it if a scientist speaks colloquially. But they don't want the answers to be silly. Last year, one kid said, "We're 11, we're not 7!"



Random Sample

Let Me Hear You Scream

The growls and screams of hardcore/metal bands like Bitterness Exhumed (pictured) sound painful, but those vocal pyrotechnics might be less damaging to the singer than you might expect. There's not much scholarly work on what happens in the throats of heavy metal singers when they perform, says musicologist Marcus Erbe of the University of Cologne in Germany. So Erbe, who has been doing field work in the heavy metal, death metal, and hardcore band scene in Germany for several years, teamed up with linguist Sven Grawunder of the Max Planck Institute for Evolutionary Anthropology in Leipzig and ear, nose, and throat physician Michael Fuchs of the Leipzig University School of Medicine to investigate.

The researchers used an endoscope to make videos of vocalists emitting growls, screams, and other standard-fare sounds of the genre. Initial results from six participants indicate that the performers—whose range rivals that of classical opera singers—produce their characteristic sounds with not only the true vocal folds but also with the vestibular folds and aryepiglottic folds, which are located higher in the larynx. And part of the desired sound comes from vibrating mucus in the singers' throats—which might also help protect their voices. Several participants have voice-intensive day jobs as counselors or teachers, but none reported any voice problems, Fuchs says. Grawunder also plans to compare the sounds with those found in, for example, unusual consonants in various languages and Tuvan throat singing in Siberia.

Metal performers push the human voice to its limits, says musicologist Michael Custodis of the University of Münster who is not part of the project. To use high-quality scientific methods to study that process "is fantastic."

Lubchenco to Leave NOAA

Marine scientist **Jane Lubchenco**, the head of the U.S. National Oceanic and Atmospheric Administration (NOAA), announced on 12 December that she will leave the job at the end of February. She plans to "return to my family and academia" in Oregon, she said in a message to NOAA staffers. "[W]onderful as Skype is for staying in touch, it is not a viable long-term arrangement!" Lubchenco has contended with some difficult challenges



Lubchenco

during her time at NOAA, including one of the world's worst oil spills in the Gulf of Mexico and the reform of major science satellite programs that were behind schedule and over budget. She also battled critics in Congress, who sank her efforts to reorganize NOAA's climate science programs and called for her firing over changes in fisheries regulation. "Our fishing community has suffered under Jane Lubchenco's leadership," said Representative John Tierney (D-MA). But Lubchenco got high marks from environmentalists for her efforts to forge a national oceans policy that put an emphasis on using science to inform policy.

<http://scim.ag/Lubchenco>

TOP 10

The Top 10 ScienceNOWs of 2012

At the end of every December, *ScienceNOW* takes a look back at some of our favorite stories of the year. These aren't necessarily the biggest scientific advances (see our **Breakthrough of the Year**, p. 1524). They're simply the funniest, wackiest, and most popular items we've run.

10 Turtle Sex—Preserved for the Ages

If anything could be more embarrassing than dying while having sex, it might be being preserved *in flagrante delicto* for millions of years so that members of an advanced species



could dig you up, gawk at you, and write a journal paper about your final romantic encounter. For a group of ancient turtles, this nightmare came true.

9 The Physics of Spilled Coffee



Here's some news you can use. Physicists have figured out how coffee spills from your mug—and how to keep it from sloshing all over your keyboard. One hint: Watch the acceleration.

8 Old Termites Blow Themselves Up To Protect the Nest

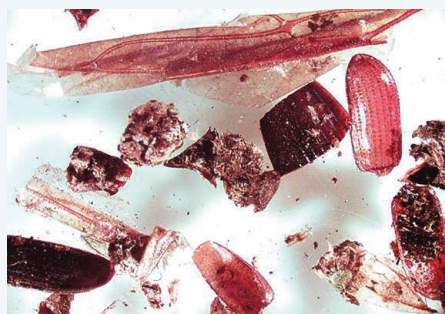
Elderly termites do not go softly into that good night. When another species of termite or a predator approaches, the termites activate explosive



crystals on their backs and go boom. Secretions released by the blast kill opponents—and save the nest.

7 Clues to Species Decline Buried In Pile of Bird Excrement

Talk about a pile of ... well, excrement. By digging into a 2-meter-deep mound of bird poop laying at the bottom of a five-story-high chim-



ney and deposited over 48 years, researchers have uncovered new clues about why the chimney swift and other species like it have begun to disappear.

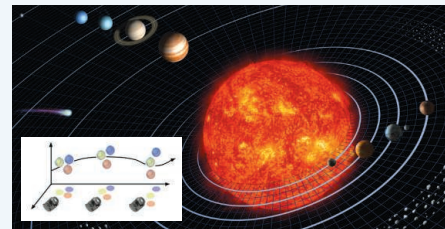
6 Landscape of Dead Bodies May Have Inspired First Mummies

Three thousand years before the ancient Egyptians began mummifying their dead, hunter-gatherers known as the Chinchorro adopted the practice in Chile's Atacama Desert. Their inspiration, according to this study, was seeing corpses rising from the sand during their daily journeys.



5 Meet 'Amasia': The Next Supercontinent

In about a hundred million years, you may be able to take a train from South America to Australia. That's because most, not all, of today's continents will be merged into a giant land-mass called Amasia. If you can't wait, flying is still your best option.

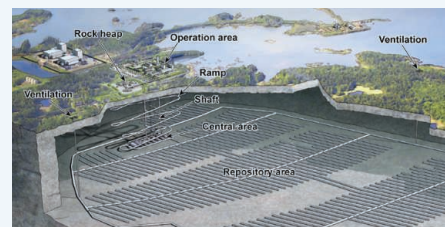


4 It's Official: Physics Is Hard

Finally, science has proved what most of us always suspected: Physics is difficult. One of the most common goals in the field—finding an equation that describes how a system changes over time—is defined as “hard” by computer theory. Just don't expect to get out of that pop quiz.

3 A Million-Year Hard Disk

Pity the humans that come across our nuclear waste repositories tens of thousands of years hence. How will they know what they are?



Enter a sapphire disk engraved with platinum that can store information for more than a million years. Now we just need to decide what language to write it in.

2 Mysterious Fairy Circles Are 'Alive'

Footprints of the gods? Signs from extraterrestrials? Namibia's so-called fairy circles have long puzzled scientists. One researcher thinks he has finally come close to solving the mystery.



1 And the number one story is ...

It's a public nuisance, and one that's been hard to fix. But now, by utilizing a bit of non-Newtonian physics, a group of college students may have hit upon a solution. One of our “silly”-est stories of the year is also our most popular. To find out what it is, visit: http://scim.ag/top10_12

U.S. FUSION RESEARCH

NIF Report Asks for More Time to Achieve Ignition

The National Ignition Facility (NIF) faces an uncertain future after its managers admitted to Congress this month that they need at least another 3 years to try to identify what has prevented the giant laser fusion lab in California from achieving ignition.

The report sharpens an ongoing debate between those who say that NIF is essential to the maintenance of the nuclear weapons stockpile and opponents who claim that NIF is a boondoggle. NIF officials believe that major cuts in the facility's \$450-million-a-year operating budget could slow progress. But obtaining enough funding could be difficult given the intense pressure to reduce domestic spending.

When NIF went into full operation in 2009, the facility's managers confidently predicted achieving ignition—a self-sustaining fusion reaction that produces excess energy—before the end of fiscal year 2012. That didn't happen, however, and this month's report, mandated by Congress, doesn't attempt to set a new goal. "At present, it is too early to assess whether or not ignition can be achieved at the National Ignition Facility," wrote Thomas P. D'Agostino, head of the National Nuclear Security Administration (NNSA), which manages NIF at Lawrence Livermore National Laboratory, in the report.

That uncertainty shouldn't count against NIF, say its supporters. "That's the nature of science. It would be absurd to build it and not use it," says Representative Zoe Lofgren (D-CA), whose district abuts the Livermore lab. But Marylia Kelley, director of the Livermore-based campaign group Tri-Valley Communities Against a Radioactive Environment, says: "NIF is actually taking money away from good science and needs to be held accountable."

NIF, which cost \$3.5 billion to build, attempts to create a burning fusion plasma by explosively compressing a small capsule of hydrogen fuel with powerful laser beams. Ignition is achieved when the fusion burn is both self-sustaining and produces more energy than the laser pulse that sparked it. A burning fusion plasma, which powers stars and H-bombs, could in theory provide clean and virtually limitless energy.

But NIF's primary goal is to help maintain America's nuclear arsenal. Weapons scientists use it to verify their computer simulations of how bombs operate and to test components for blast-hardness. Ignition is crucial for both energy and weapons goals.

NIF scientists have relied heavily on work with earlier lasers and computer modeling to develop a design for the target—a peppercorn-size sphere full of frozen hydrogen isotopes—and the shape of the laser pulse needed to implode it. Those models predicted that NIF should already be producing ignition. And while NIF's laser, diagnostic instruments, and target fabrication have met or exceeded specifications, the physics of the implosions remains a puzzle.

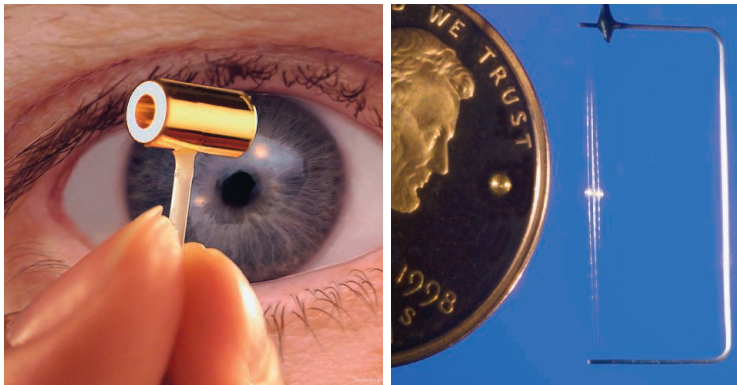
"The disagreement between NIF experimental data and codes and models reflects an inadequate understanding of key physics issues," the report says. "Mother Nature kind of won on this one," says Mary Hockaday, deputy associate director for weapons physics at Los Alamos National Laboratory in New Mexico and one of the lead authors of the report.

NNSA has proposed a 3-year program to investigate those key physics issues and develop models that are better able to predict what is actually happening. "NIF was sized to do it, and we still believe it's possible," says Christopher Deeney, assistant deputy administrator for stockpile stewardship at NNSA.

NNSA plans to request funding for the new program "not quite at the level in FY2013, but not down significantly," Deeney says. But the current proposed level may not hold up. Senator Dianne Feinstein (D-CA), chair of the Senate Appropriations Subcommittee on Energy and Water Development, writes in an e-mail that "the committee must be assured of substantial progress toward NIF's

goal of achieving stockpile stewardship or the \$450 million annual cost of operations will be difficult to justify."

The NNSA report says the new program should explore alternatives to the indirect drive approach now used at NIF, in which the laser beams heat a gold cylinder the size of a pencil eraser that surrounds the target. Researchers at the University of Rochester's Laboratory for Laser Energetics and the Naval Research Laboratory in Washington, D.C., have spent decades working on the alternative approach—direct drive—in which the laser beams shine directly onto the target capsule. "Nine times as much energy ends up on the capsule containing the fuel," says Robert McCrory, director of the Rochester lab. The other proposed back-up is a technique devised with Sandia National Laboratory's Z machine that uses huge electrical pulses to crush fusion fuel magnetically.



Two routes to ignition. One approach aims a laser directly at a target (right), while another uses them to first heat a tiny gold container.

"Indirect drive is still the focus," Deeney says. "If indirect drive encounters problems, we can change horses. But there is no evidence the other approaches won't have the same problems."

The NNSA report assures legislators that the failure, thus far, to achieve ignition will not undermine the safety of current weapons. It says scientists can use results from the underground testing program (which ended in 1992) as well as data from monitoring the weapons themselves.

But the current problems with models and simulations limit the extent to which weapons scientists can use them to modify existing weapons. Critics point to that fact in arguing that NIF's true goal is to keep weapons designers usefully employed in case they are needed to develop new weapons. "You do not need a giant laser to maintain the stockpile we have," Kelley asserts.

—DANIEL CLERY

CAREERS

An *Annus Horribilis* for Anthropology?

It's been a rough year or so for anthropology. Back in fall 2011, Florida Governor Rick Scott proclaimed that his state didn't need any more anthropologists, and that public money would be better spent educating scientists (never mind that anthropology is the *science* of humanity). Then in January, a study announced that graduates with bachelor's degrees in anthropology faced a particularly bleak unemployment rate. In August and October, two high profile business reports—from *Kiplinger* and *Forbes*—ranked anthropology as the worst major for both employment and starting pay.

Some anthropologists responded with gal-lows humor. "We're #1!" anthropologist Jason Antrosio of Hartwick College in Oneonta, New York, posted on his blog, Living Anthro-

bad juju was released in January by the Center on Education and the Workforce at Georgetown University. Using 2009 and 2010 data from the U.S. Census Bureau, researchers found that the unemployment rate among recent graduates with bachelor's degrees in anthropology and archaeology was 10.5%, surpassed by only majors in architecture, philosophy/religious studies, certain arts, and information systems. Anthropology majors who did get jobs were among the lowest paid, starting at \$28,000 a year. *Kiplinger* and *Forbes* used this study plus salary data from PayScale.com to make their own rankings.

Anthropologists point out that high unemployment is common in fields like anthropology and psychology where professionals need graduate degrees, and many undergrad

and apply that knowledge to current issues." At the University of Memphis in Tennessee, for example, a remarkable 87% of students with new master's degrees get hired, usually within 6 months, at nonprofit organizations, schools, government, business, and in healthcare, says Ruthbeth Finerman, chair of anthropology. She credits their success partly to an outreach program that puts students in local jobs and internships.

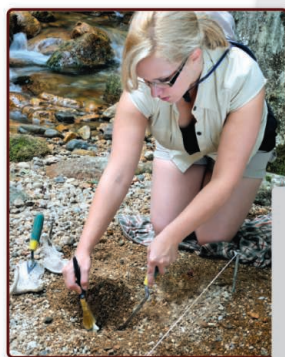
Programs that track students have found that even undergrads get jobs that use anthropological skills, but in diverse fields. ECU grads have had "good success" getting jobs in forensics, at historic museums, state zoos, and as Web designers, Mathews says. Some departments now require capstone or practicum courses, in which seniors review their skills and draft career plans. At the University of North Carolina (UNC), Charlotte, students are encouraged to get hands-on experience at an internship and to take statistics and other skills-based courses, says Janet Levy, UNC Charlotte's anthropology department chair.

AAA has also geared up to help, hiring a fellow to focus on career issues, and offering a workshop for undergrads at its annual meeting this year. "Universities should become more

realistic in what their [undergraduate] students are going to be doing after they get a degree," says workshop leader Carol Ellick, the author of a book on the transition from college to a career in anthropology. "A lot of professors still don't acknowledge the applied side of things because they never worked in the private sector or government."

Undergraduate anthropology can't be billed as "preprofessional training," says Allan Gilbert, chair of sociology and anthropology at Fordham University in New York City. But anthropologists are learning to market what they do best: "Anthropology will teach [students] about diversity in the world, why people vary in custom and in appearance, where humans came from, and how humans have colonized most of the Earth's surface," he says. "If that, plus obtaining the skills of thinking and clear expression, doesn't prepare a student to live in an increasingly multicultural world, it's unclear to me what will."

—ANN GIBBONS



Rick Scott, Florida Governor, Says Of Anthropologists, 'We Don't Need Them Here'
—The Huffington Post

Rick Scott no fan of anthropology

—Herald-Tribune

The 10 Worst College Majors

No. 1: Anthropology And Archeology —Forbes

WORST COLLEGE MAJORS FOR YOUR CAREER

1. Anthropology

—Kiplinger

Scott: Anthropology and journalism don't pay, and neither do capes

—Orlando Sentinel

pologically. "Anthropology is the worst major for immediate career, but anthropology is the major most likely to change your life."

Others organized workshops. At the annual meeting of the American Anthropological Association (AAA) in San Francisco last month, students and chairs of anthropology departments met in separate sessions to discuss job prospects and how to prepare students. Most said the bad press wasn't fair, noting that the situation is very different for bachelor's degree- and advanced degree-holders. Many added that even undergraduate degrees in anthropology prepare students well for many jobs (and life), but those jobs are hard to track because they seldom are listed as "anthropologist" or "archaeologist." But the rankings gave new urgency to educators to "think seriously about the skill-set we are or are not imparting to the next generation of anthropologists," says Terrence Deacon, chair of anthropology at the University of California, Berkeley.

The study that triggered the avalanche of

majors are headed for medicine, law, or graduate school. Indeed, few of the annual 10,000 anthropology baccalaureates expect to find jobs as "professional" anthropologists, unlike the 73,000 engineering grads or the 40,000 in computer science. "We tell our undergraduates that there are no jobs out there labeled 'anthropologist,'" says Holly Mathews, director of the undergraduate program in anthropology at East Carolina University (ECU) in Greenville, North Carolina. Comparing the value of degrees in anthropology and engineering is "less like comparing apples to oranges than comparing aardvarks to toaster ovens," AAA President Leith Mullings wrote to *Forbes*.

The roughly 1800 new M.S. and Ph.D. graduates in anthropology and archaeology seem to face a rosier job picture, according to the U.S. Bureau of Labor Statistics. The bureau predicted a 21% increase in anthropology jobs from 2010 to 2020, because "more anthropologists will be needed to research human life, history, and culture,



METEORITICS

Sutter's Mill Meteorite Produces Mother Lode of Research

At about 8 a.m. on Sunday, 22 April, a fiery, minivan-sized boulder from space streaked across the sky over California and Nevada, setting off a sonic boom detected as far away as Washington state. Witnesses stopped to photograph the fireball, which exploded with the force of 4 kilotons of TNT during its descent. Most of it vaporized, but the remaining fragments showered the towns of Coloma and Lotus in California with small black meteorites.

The Sutter's Mill meteorite—named for the nearby site of the 1848 gold strike that triggered the California gold rush—immediately caught the attention of astronomer Peter Jenniskens of the SETI Institute and NASA Ames Research Center in Mountain View, California. An experienced meteorite hunter who had led collection efforts in Sudan, Jenniskens sprang into action, organizing a consortium of scientists to find and analyze the fragments. This week, he and 69 co-authors present their findings on page 1583. After myriad tests including x-rays and isotopic analyses, they conclude that the meteorite is the most pristine sample yet collected of a rare type of carbonaceous chondrite, and possibly a sneak preview of a primitive asteroid to which NASA might one day send astronauts.

Scientists are fascinated by asteroids and comets because they reflect the original chemical makeup of the solar system when it formed roughly 4.5 billion years ago, says Richard Binzel, a planetary scientist at the Massachusetts Institute of Technology in Cambridge who was not involved in the new study. Much of that chemistry can be lost in the reactive atmosphere of Earth—

particularly volatile carbon-based compounds such as amino acids. Even breathing on a meteorite can destroy its fragile chemicals or contaminate it, Binzel says, so it is vital to recover meteorites as quickly as possible. Jenniskens's efforts to track down the Sutter's Mill meteorites quickly were “exemplary,” he says.

Hoping to collect samples of the meteorite before the next rainfall, on Tuesday after the meteorite's first sighting, Jenniskens jumped in his car and drove to Lotus. Marc Fries, a meteoriticist at the Planetary Science Institute in Tucson, Arizona, had helped Jenniskens pinpoint the location of the fallen meteorites with weather radar—a technique that Fries pioneered and that is enabling faster recovery of meteorites worldwide. By the time Jenniskens arrived, professional meteorite hunter Robert Ward had already made the first find: a 5.6-gram chunk along the roadside. After a frustrating initial search in a state park, Jenniskens looked down and spotted a second meteorite in the parking lot. At 4 grams, it had been crushed by a car tire but was still largely intact.

Back in the lab, cosmic mineralogist Michael Zolensky of NASA's Johnson Space Center in Houston, Texas, broke the meteorites apart and looked at them with an electron microprobe. Despite its abuse, the meteorite that had been run over still contained chestnut-brown crystals of oldhamite, a rare mineral that sparkles like fool's gold and is thought to have originated in the solar nebula when the solar system formed. Oldhamite is highly reactive. “Had we polished the meteorite with alcohol or water, it would have been gone,” Zolensky says. Isotopic analysis of the samples showed that the Sutter's Mill meteorite

Treasure hunt. Peter Jenniskens and colleagues from SETI and NASA search high and low for meteorites.

was a rare CM-type carbonaceous chondrite that contains largely unaltered materials from the dawn of the solar system. Few such meteorites have been recovered, and none in time to detect fragile compounds like oldhamite.

On Wednesday it started to rain. However, the search for meteorites continued for. Volunteers continued to comb the terrain into the 40°C—plus heat of late summer, negotiating with locals to gain access to their property. Jenniskens and meteoriticist Qing-zhu Yin of the University of California, Davis, held town meetings to teach people how to identify the deep-black meteorites by their iridescent fusion crust, and to handle the meteorites with aluminum foil instead of touching them. Alex Wolfgram was one local they didn't catch in time: When he found a small meteorite on a sandy beach while kayaking, he picked it up and put it in his wet life jacket. Later, he used the fragment to propose to his girlfriend, a geologist.

Overall, the team recovered 77 meteorites with a total mass of 943 grams. As more pieces came in, it became clear that the meteorite was a composite of bits and pieces of different asteroids that collided in space. The presence of minerals that form under extremely hot, dry, reducing conditions, like oldhamite, and compounds that form in cold, wet conditions, like amino acids, show that different parts of the meteorite had very different thermal and chemical histories, Zolensky says. The team also recently identified diamonds in the meteorite, he says, “which do not belong in either of these environments.”

From photographs and videos of the fireball, Jenniskens determined that the asteroid entered Earth's atmosphere at twice the typical speed—a record 26.8 km per second—and at a low angle relative to Earth's equator. Combining that information with estimates of how long the fragment had been exposed to cosmic rays in space, he calculated that it came from the inner region of the asteroid belt between Jupiter and Mars, and had followed an orbit similar to that of Jupiter-family comets. The use of available technology to pinpoint where the meteorite probably came from and quickly identify where it fell is “a huge success,” Binzel says. “The scientific importance of those fresh samples cannot be overstated.” Now, scientists need to get samples of the type of asteroid that the Sutter's Mill meteorite came from by going to space, he says. “If we can link the meteorites to their origins in space, it will put a gold mine of research into a solar system context.” —EMILY UNDERWOOD

CREDITS: NASA/ERIC JAMES (INSET) NASA/COURTESY OF DR. PETER JENNISKENS



No Lake Mud for Curiosity Rover to Investigate?

A pile of dust? Local winds may have built the central mound of Gale crater from passing dust.

To Mars scientists, Gale crater's central mound seemed an irresistible twofor: an environmental record from when the once-water-rich planet slid into hyperaridity, plus the prospect of analyzing sediments that might have been laid down in an ancient lake. That would be the ideal place to find remains of any early martian life.

But at the meeting, a pair of researchers probing the internal structure of the mound for clues to its formation put a damper on these hopes. "We see nothing in the geometry of the mound that would suggest [lake sediments] in it," says planetary scientist Kevin Lewis of Princeton University.

Lewis and planetary scientist Edwin Kite of the California Institute of Technology in Pasadena and colleagues used images from the Mars Reconnaissance Orbiter (MRO) with a resolution of 1 meter per pixel. That's the highest resolution yet available, high enough to make out the thin layers of sediment exposed around the 5-kilometer-tall mound. They also determined the mound's topography using stereo MRO images made by combining pairs of images taken from slightly different points in orbit. Taken together, the images allowed the researchers to measure the inclination of the sediment layers in the mound.

What Lewis and Kite found bears little resemblance to a deposit formed at the bottom of a lake. Lake sediments are deposited as flat-lying beds. But the mound's sediment layers are consistently inclined by 2° to 4°, they reported. At the seven outcrops examined around the mound, the beds slope away from the center of the mound, echoing the mound's present-day shape, not the shape of a lake bottom. And when partially eroded beds exposed around the mound are

extended radially outward, none reaches the crater wall. All of this seriously challenges the leading explanation for the mound: that Gale crater was once filled to the brim with sediment that has largely blown away to leave the mound.

Searching for another way that Gale's mound could have formed, Kite and Lewis conclude that the wind did it. In their scenario, the mound "rose from nothing on the crater floor," Lewis says. Gale lies in an especially dusty region of the dustiest planet known. Once the huge impact billions of years ago raised the Gale crater rim, solar heating of the crater floor would drive winds up over the rim during the day, and later cooling would drive winds down from the rim at night. These topographic winds would clear dust from the outer parts of the crater floor, but a stagnant region in the center—where the winds mainly blow up or down—would trap any dust delivered by inward winds.

If that's the way it worked, Gale mound would be a very high pile of dust. "There must have been more going on," Lewis says; perhaps ground water altered and welded together minerals near the bottom of the mound. "But it's a great base hypothesis to explain most of the deposit," he says.

No one has been entirely happy with the filling and partial removal idea, so the topographic wind hypothesis "is intriguing," says planetary scientist James Bell of Arizona State University, Tempe. Even so, he adds, "I don't think it's a slam dunk. It looks like a variety of processes were at work there." The mound's origins may prove complicated, but planetary geologist James Head of Brown University thinks Curiosity has the required tools: "It's going to be exciting moving up and down those slopes and sorting this out."

Tying Megaeruptions To a Mass Extinction Long After the Fact

To incriminate a global catastrophe in the extinction of a wide swath of the biosphere, you need precise dates for two events: the catastrophe—say, an asteroid impact or volcanic eruption—and the mass extinction. At the meeting, geochronologists who measure the passage of time in the steady ticking of radioactive decay presented convincing evidence that massive eruptions at the opening of the Atlantic Ocean 201 million years ago drove the mass extinction that cleared the way for the rise of the dinosaurs.

The dating—by Terrence Blackburn of the Carnegie Institution for Science in Washington, D.C., and colleagues—was impressively precise. For minerals from the end of the Triassic period, 201 million years ago, the researchers reported ages to three decimal places with a 1-sigma error of about 30,000 years, just 0.015% of the ages. That kind of precision takes careful measurements of the amounts of the radioactive element of interest and the product of its decay. That's quite a feat in mineral grains that have been ravaged for hundreds of millions of years by both the environment and their own radioactivity.

Radiometric dating has been in use for half a century, but in recent years a National Science Foundation program called EARTH-TIME has prodded some improvements. New laboratory procedures and data analysis software developed through EARTHTIME-sponsored interlaboratory collaborations have helped reduce uncertainties in uranium-lead radiometric dating. And uranium-lead labs have adopted a sample pretreatment that effectively dissolves away parts of the mineral that has lost some of its lead because of radiation damage, leaving only mineral that has locked in all the lead it was endowed with.

Near the end of the Triassic period, millions of cubic kilometers of magma spewed from the crack that split the supercontinent Pangaea in two and started the opening of the Atlantic Ocean. Debris from the eruptions might have chilled the climate or poisoned the environment, triggering the extinction. But previous dating had had the extinction coming before the first volcanic outburst, not at the same time.

So Blackburn and his colleagues used the latest uranium-lead techniques to date volca-

nic samples from seven sites on the East coast of North America and one site in Morocco. They dated the end-Triassic extinction to 201.562 ± 0.016 million years ago (subject to change in peer review). Adding in dating of sediments surrounding eruption deposits by using astronomical cycles, they could correlate the Moroccan record to the North American record, placing the extinction at the first of three eruption pulses within the small dating errors. And those errors have gotten so small that no one is disputing that the Atlantic-opening megaeruptions somehow did in enough critters to unleash the dinosaurs.

Buildup to Quakes Spied in Both Model And Real World

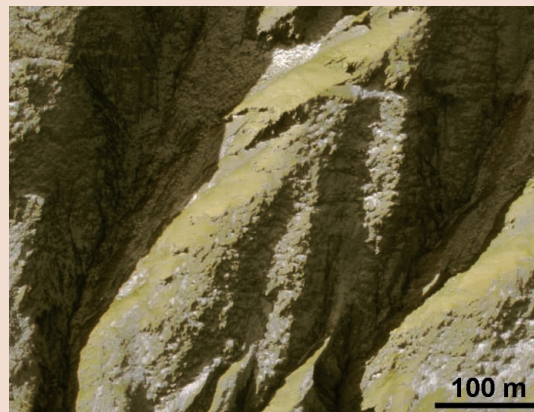
Seismologists want to know how earthquakes work. At the meeting, researchers reported one small step toward that goal. Peering beneath the sea floor east of Japan and into the innards of a model of a fault like the big one that runs there, they saw slow failure of a fault—so slow that the ground never shook—preceding large, ground-shaking earthquakes. These observations make clear how slow slip events around the Pacific Ring of Fire are loading stress drip by drip onto dangerous faults, eventually driving them to generate great earthquakes.

In a seismology session, Harmony Colella of Miami University in Oxford, Ohio, and colleagues reported seeing slow slip events transfer stress in a fault model. In their relatively simple computer simulation, the fault is assigned four properties—such as how friction on a fault varies with slip behavior—that were derived from lab or real-world observations. Like the fault off the coast of Japan, the model's fault descends into deeper and, therefore, hotter regions. As a result, the upper part of the model fault is cold enough to remain locked tight, so it breaks in quakes. The middle or transition zone slips in slow slip events, and the deepest part is always slipping downward as the ocean plate dives into the mantle.

After running hundreds of thousands of simulations, Colella says, "it was remarkable how well the model matched

Snapshots From the Meeting >>

Drying out Mars. Another volley in the ongoing debate over water on Mars argues that water is not cutting gullies in the present-day martian surface. Changes in the appearance of martian gullies from year to year first reported in 2006 had suggested that a liquid—presumably water unleashed from ice by summer's heat—was intermittently flowing down gullies. But at the meeting, planetary geologist Colin Dundas of the U.S. Geological Survey in Flagstaff, Arizona, and his colleagues reported that the sharp-eyed Mars Reconnaissance Orbiter has peered repeatedly into the deepest shadows of martian winter and seen only carbon dioxide (CO₂) frost (aka dry ice) at work. Everything from fine debris to boulders is seen to move downhill when CO₂-frost begins to disappear, and gully activity is concentrated in winter when water is still frozen solid, not in summer. "Current activity seems to be driven by CO₂," Dundas says, though water may well have played a role in cutting gullies in the geologic past under warmer climates.



Dry erosion. In martian shadow, CO₂ frost (lighter patches), rather than water, can mobilize dark debris in channels.

Making a bigger Big One. The burgeoning subsurface injection of wastewater—mostly derived from "fracking" for oil and gas—has been setting off sizable earthquakes from New Mexico to Arkansas to Ohio (*Science*, 23 March, p. 1436). But the biggest suspected induced quake—the magnitude-5.7 Prague quake that struck central Oklahoma in November 2011—came long after the start of nearby injection. That cast doubt on any link to deep disposal. The 5.7 Prague was the second of three related quakes, however, and at the meeting, seismologist Katie Keranen of the University of Oklahoma, Norman, and colleagues reported that the first fault segment to rupture was less than 200 meters from two active injection wells and broke to the depth of the injection. They think known geological barriers delayed the quake by temporarily holding back the injected wastewater. The large size of the Prague quake relative to the small volume of injected wastewater suggests deep disposal can set off large quakes that might not have occurred naturally for centuries.

—R.A.K.

the observations [of slow slip] without fine-tuning things or making a lot of assumptions." The model's slow slip events even reversed as they progressed through the transition zone, as observed, going faster in the opposite direction. Disconcertingly, the model's transition zone also slipped and released energy in earthquakes that ruptured the locked zone above it. Because the transition zone is closer to the coast, that would mean more damage on nearby land.



Post-buildup. Slow slip built stress on the fault that failed in the 2011 quake.

At a tectonophysics session, geophysicist Yoshihiro Ito of Tohoku University in Sendai, Japan, and colleagues reported that the fault off Japan behaved much

like Colella's model fault. They used observations of deforming crust from three kinds of instruments to measure slow slip offshore before the great quake. In hindsight, the Japanese group detected two slow slip events, each of which released about as much energy in weeks as the quakes did in seconds.

The two slow slip events appeared to transfer stress onto adjacent fault patches, triggering those patches to rupture in earthquakes. Interestingly, the second slow slip event came in February 2011 and triggered a magnitude-7.3 quake just 2 days before the magnitude 9.0 quake. So, slow slip triggered the megaquake's largest foreshock. And as in the modeling, the patch of fault traversed by slow slip also failed in the great quake. That's potentially bad news for the U.S. Pacific Northwest, Colella says, which is awaiting its own offshore great quake.

—RICHARD A. KERR

THE DISCOVERY OF THE HIGGS BOSON

NO RECENT SCIENTIFIC ADVANCE HAS generated more hoopla than this one. On 4 July, researchers working with the world's biggest atom smasher—the Large Hadron Collider (LHC) in Switzerland—announced that they had spotted a particle that appears to be the long-sought Higgs boson, the last missing piece in physicists' standard model of fundamental particles and forces. The seminar at which the results were presented turned into a media circus, and the news captured the imagination of people around the world. "[H]appy 'god particle' day," tweeted will.i.am, the singer for pop group The Black Eyed Peas, to his 4 million Twitter followers.

Online

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S For an expanded version of this section, with podcast, video, links, and more, see www.sciencemag.org/special/btoy2012 and sciencecareers.org.

Yet, for all the hype, the discovery of the Higgs boson easily merits recognition as the breakthrough of the year. Hypothesized more than 40 years ago, the Higgs boson is the key to physicists' explanation of how other fundamental particles get their mass. Its observation completes the standard model, perhaps the most elaborate and precise theory in all of science. In fact, the only big question hanging over the advance is whether it marks the beginning of a new age of discovery in particle physics or the last hurrah for a field that has run its course.

The Higgs solves a basic problem in the standard model. The theory describes the particles that make up ordinary matter: the electrons that whiz around in atoms, the up quarks and down quarks that make up the protons and neutrons in atomic nuclei, the neutrinos that are emitted in a type of radioactivity, and two sets of heavier cousins of these particles that emerge in particle collisions. These particles inter-

act by exchanging other particles that convey three forces: the electromagnetic force; the weak nuclear force, which spawns neutrinos; and the strong nuclear, which binds quarks.

But there's a catch. At first blush, the standard model appears to be a theory of massless particles. That's because simply assigning masses to the particles makes the theory go haywire mathematically. So mass must somehow emerge from interactions of the otherwise massless particles themselves.

That's where the Higgs comes in. Physicists assume that empty space is filled with a "Higgs field," which is a bit like an electric field. Particles interact with the Higgs field to acquire energy and, hence, mass, thanks to Albert Einstein's famous equivalence of the two, encapsulated in the equation $E = mc^2$. Just as an electric field consists of particles called photons, the Higgs field consists of Higgs bosons woven into the vacuum. Physicists have now blasted them out of the vacuum and into brief existence.

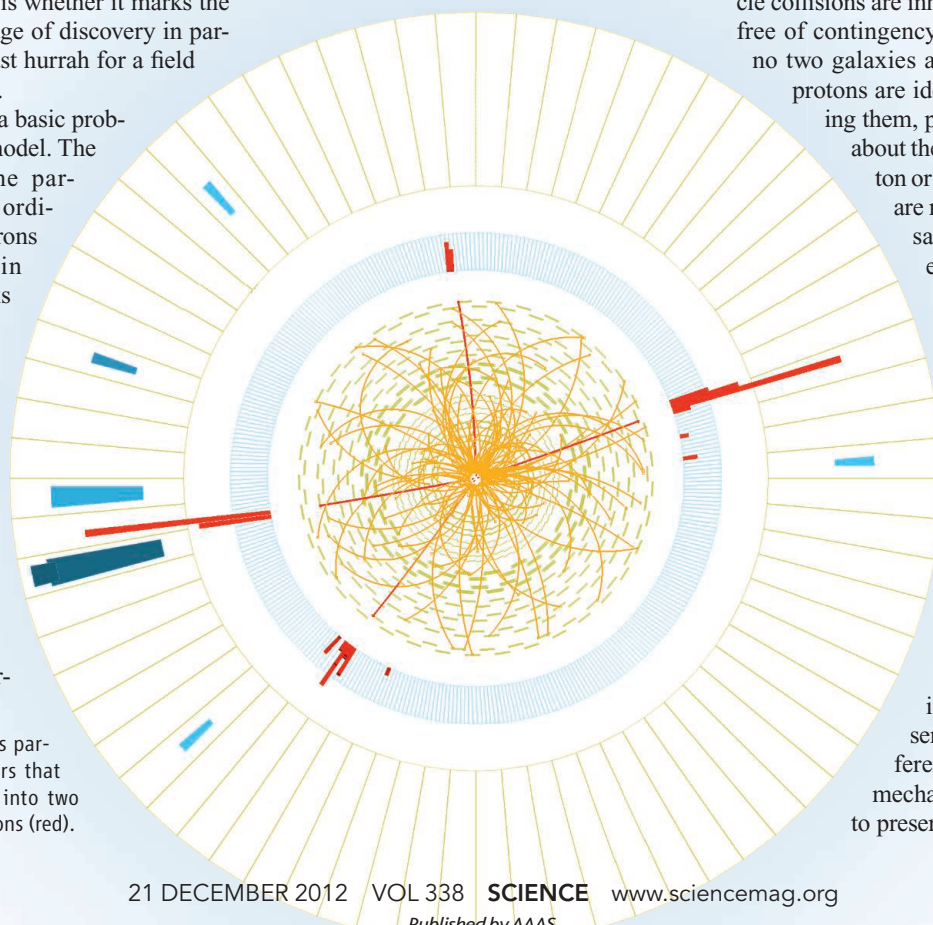
That feat marks an intellectual, technological, and organizational triumph. To produce the

Higgs, researchers at the European particle physics laboratory, CERN, near Geneva, built the \$5.5 billion, 27-kilometer-long LHC. To spot the Higgs, they built gargantuan particle detectors—ATLAS, which is 25 meters tall and 45 meters long, and CMS, which weighs 12,500 tonnes. The ATLAS and CMS teams boast 3000 members each. More than 100 nations have a hand in the LHC.

Perhaps most impressive is the fact that theorists predicted the existence of the new particle and laid out its properties, right down to the rates at which it should decay into various combinations of other particles. (To test whether the particle really is the Higgs, researchers are measuring those rates now.) Physicists have made such predictions before. In 1970, when only three types of quarks were known, theorists predicted the existence of a fourth, which was discovered 4 years later. In 1967, they predicted the existence of particles that convey the weak force, the W and Z bosons, which were found in 1983.

Particle theorists offer various explanations of their knack for prognostication. Particle collisions are inherently reproducible and free of contingency, theorists say. Whereas no two galaxies are exactly the same, all protons are identical. So when smashing them, physicists need not worry about the peculiarities of this proton or that proton because there are none. Moreover, theorists say, in spite of its mathematical complexity, the standard model is conceptually simple—a claim that nonphysicists might not buy.

The standard model ultimately owes its predictive power to the fact that the theory is based on the notion of mathematical symmetry, some theorists say. Each of the three forces in the standard model is related to and, in some sense, necessitated by a different symmetry. The Higgs mechanism itself was invented to preserve such symmetry while



Pieced together. In this particle collision, it appears that a Higgs boson decays into two electrons and two positrons (red).

giving mass to force-carrying particles like the W and the Z. Simply put, symmetry arguments are powerful predictive tools.

No matter the reason for particle physicists' predictive prowess, with the Higgs boson apparently in the bag, they have no similar prediction to test next. They have plenty of reason to think the standard model is not the final word on fundamental physics. The

theory is obviously incomplete, as it doesn't incorporate the force of gravity. And the theory itself suggests that interactions between the Higgs and other particles ought to make the Higgs hugely heavy. So physicists suspect that new particles lurking in the vacuum may counteract that effect. But those arguments aren't nearly as precise as the one necessitating the Higgs boson.

In fact, scientists have no guarantee that any new physics lies within the reach of the LHC or any conceivable collider. The standard model could be all of the inner workings of the universe that nature is willing to reveal. The discovery of the Higgs is a breakthrough. Will particle physicists ever score a similar breakthrough again?

—ADRIAN CHO

A HOME RUN FOR ANCIENT DNA

Two years ago, paleogeneticists made our short list for Breakthrough of the Year for publishing the complete sequence of the nuclear genome of the Neandertals. In 2011, the same lab shared our spotlight for piecing together the genome of the Denisovans, an archaic human that lived in Siberia at least 50,000 years ago. But those ancient DNA sequences and others were blurry snapshots next to the high-resolution genomes that researchers can now sequence from living people. Much of the fragile DNA from fossils is degraded into single strands that automatic sequencers can't copy. Researchers were resigned to deciphering only parts of the code of ancient genomes, whether from archaic humans, animals, or pathogens.

This year, however, a persistent postdoc developed a remarkable new method that enabled his team to revisit the Denisovan DNA and sequence it 31 times over. The resulting genome, of a girl who lived in Siberia's Denisova Cave, reveals her genetic material in the same sharp, rich detail that researchers typically get from the DNA of living people. This technological feat promises to give a major boost to the field of ancient DNA, as researchers begin to apply the method to other samples and species.

Ancient DNA researchers typically have adapted the tools used to sequence DNA from living humans, which start with samples of double-stranded DNA. But ancient DNA usually breaks into single strands. So postdoc Matthias Meyer at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, set out to sequence single-stranded ancient DNA from scratch. He failed at first, but then managed to bind special molecules to the ends of a single DNA strand, holding it in place for sequencing. As a result, using only 6 milligrams of bone from the Siberian girl's pinky finger, Meyer and colleagues were able to copy 99.9% of her genome at least once and 92%



Single-minded. Postdoc Matthias Meyer (*above*) developed a new method to prepare single strands of ancient DNA; the technique gave researchers an unprecedented view of an ancient girl's genome.

of the genome 20 times—the benchmark for reliably identifying nucleotide positions.

The results confirmed that Denisovans interbred with the ancestors of some living humans; people living in parts of island Southeast Asia have inherited about 3% of their nuclear DNA from Denisovans. The genome literally offers a glimpse of the girl, suggesting that she had brown eyes, brown hair, and brown skin. It also allowed the team to use DNA to estimate that the girl died between 74,000 and 82,000 years ago—the first time researchers had used genomic information to date an archaic human. The high quality of the genome gives researchers a powerful new tool to fish for genes that have recently

evolved, providing a “near-complete” catalog of the handful of genetic changes that separate us from Denisovans, who were close kin to Neandertals.

These details are all the more remarkable because the Denisovans are so poorly known from fossils: Only a tiny scrap of finger bone and two molars have been reliably assigned to them so far. In contrast, the Neandertals are known from hundreds of fossils but from a much less complete genome.

Neandertal experts may catch up soon. Meyer and colleagues have been trying “Matthias's method” on fossil samples that previously failed to yield much DNA. A detailed Neandertal genome comparable to the Denisovan one is expected in 2013.





ITALIAN QUAKE VERDICTS RATTLE RESEARCHERS

Can scientists risk talking publicly about risk—especially when lives are on the line? It's a question that many researchers began asking this year as Italian prosecutors pressed manslaughter charges against four scientists, two engineers, and a government official accused of conducting superficial analyses and making misleading public statements about earthquake hazards in the days before a deadly 2009 temblor struck the city of L'Aquila, killing more than 300 people. In October, each of the seven defendants was found guilty and sentenced to 6 years in prison; all are appealing the convictions, a process that could take years.

The verdicts shocked and enraged many researchers—and prompted them to revisit a long-standing challenge: how to communicate with the public and policymakers about risk, especially in technical areas with high uncertainty and the potential for great loss of life. From bioterrorism and disease outbreaks to hurricanes and earthquakes, researchers are called upon by government officials to help forecast the probability of dangerous events and devise plans to keep the public safe. They often struggle to translate nuanced statistical models into plainspoken, practical advice. Should a 40% probability that an event might occur make it “low-risk” in common parlance, for instance—or should that be a “medium” risk? And what difference would that make to a person trying to decide whether to flee an oncoming storm, or a government official trying to prepare for a possible bioterror attack?

Until now, the answers to such questions were largely academic, or at least low-risk from a legal perspective. After the Italian verdicts, however, some scientists worry that the words they utter might land them in prison. “I’m afraid that many scientists are learning to keep their mouths shut,” earth scientist Thomas Jordan of the University of Southern California in Los Angeles, told *Science* earlier this year. “This won’t help those of us who are trying to improve risk communication between scientists and the public.”

Some science groups are working to make sure researchers don’t go silent. National academies of science in Europe, for example, are collaborating on efforts to extract lessons from the L'Aquila case, with an eye toward heading off similar legal jeopardy elsewhere. “Probability-based statements are per se fraught with uncertainty,” the French and German academies noted in a statement earlier this year. But “scientists cannot—and should not—absolve themselves” of the responsibility to communicate clearly, it added. The risk that scientists ultimately decide to say nothing, such efforts suggest, may be the greatest risk of all.

—DAVID MALAKOFF

GENOMIC CRUISE MISSILES

This year, genome engineers got their hands on some potentially powerful new tools that promise to put the modification of DNA within easy reach of biologists studying a variety of organisms, including yeast and humans. One of these tools, called TALENs (for “transcription activator–like effector nucleases”), can destroy or alter specific genes in zebrafish, *Xenopus* toads, and livestock. A TALEN is a protein that cuts DNA in specific places, and the ensuing repair modifies the target gene. One group of researchers used the technique to create a miniature pig useful for studying heart disease. Others are modifying the genomes of rats, crickets, and even human cells from patients with disease. Crystal structures of these effector proteins attached to DNA have revealed how the proteins find their targets. And at least three teams have come up with a way to make many of these proteins fast and cheaply. This progress has prompted more investigators to give this approach a try.

Such a boom in genome engineering was unthinkable just a few years ago. For most higher organisms, changing or deleting DNA has generally been a hit-or-miss proposition. Researchers could



Model porker. Researchers used TALENs to make pigs useful for studying heart disease.

not readily control where an added gene would insert itself into a genome or which DNA they delete in so-called knockout experiments. As a result, pinpointing what specific genes do and correcting disease genes in people have posed major challenges.

A decade ago, a new technology called zinc finger nucleases provided a way to target specific genes. Researchers leaped to develop this tool. But zinc fingers proved difficult to make, and one company holds all the key patents. So excitement swelled again in

2009, when two teams discovered a one-to-one correspondence between the repetitive regions of transcription activator–like effector proteins and the DNA bases they attach to, thus providing a new way to target genes. In 2012, studies drove home that TALENs work as well as zinc fingers do but are far easier and cheaper to make. Some researchers now think TALENs will become standard procedure for all molecular biology labs.

Meanwhile, another gene-targeting technology is beginning to make a name for itself. One drawback of zinc finger nucleases,



TALENs, and another genome-editing tool called meganucleases is that they must be reengineered for each new DNA target. These proteins have two parts: the DNA targeting section and the DNA-cutting section. The new technology substitutes RNA—which is simpler to make than a piece of a protein—for the DNA targeting section. It also makes

use of a bacterial protein called Cas9, which is part of a natural bacterial defense system called CRISPR, to do the cutting.

Researchers have shown in a test-tube that they can combine these two RNAs into a single one that both matches the DNA target and holds Cas9 in place. Using this system, they were able to cut specific target DNA,

demonstrating the potential of Cas9 to work like TALENs. Now, those researchers are trying this approach in organisms other than bacteria, and other genome engineers are quite excited about their prospects, suggesting that it may one day challenge zinc finger nucleases and TALENs as the core genome engineering technology.

CRASH PROJECT OPENS A DOOR IN NEUTRINO PHYSICS

Sometimes it's not the result itself so much as the promise it holds that matters most. This year, physicists measured the last parameter describing how elusive particles called neutrinos morph into one another as they zip along at near-light speed. And the result suggests that in the coming decades neutrino physics will be every

bit as rich as physicists had hoped—and may even help explain how the universe evolved to contain so much matter and so little antimatter.

Born in certain nuclear interactions, neutrinos come in three types or flavors that change into one another in so-called neutrino oscillations. The rates and extents to which the flavors mix depend on six parameters: the three differences between the neutrinos' masses, and three "mixing angles." In March, the 250 researchers with the Daya Bay Reactor Neutrino Experiment in China reported that last unknown parameter, the mixing angle known as θ_{13} (pronounced "theta one three"), equals 8.8° , give or take 0.8° .

The result itself is remarkable, as it's not every year that physicists measure a new fundamental parameter. The real excitement, however, stems from the result's broader implications. The measurement proves that all three mixing angles are greater than zero. That fact, in turn, implies that the oscillations of antineutrinos might differ from those of neutrinos, something that would not be possible had θ_{13} equaled zero.

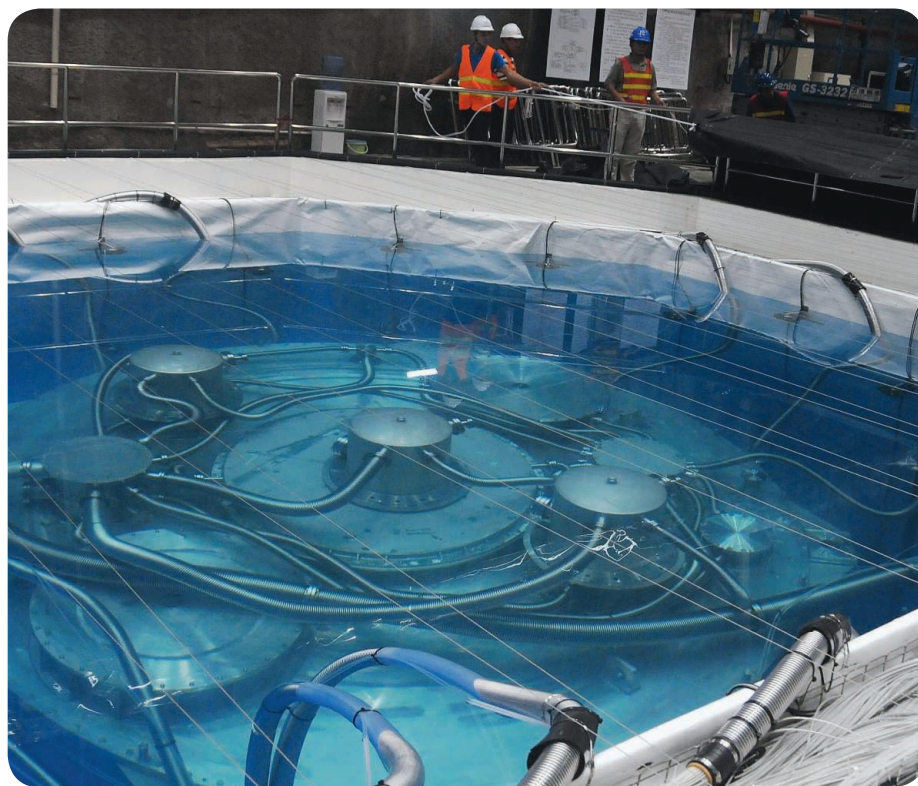
That's a big deal. Such a difference would be an example of an asymmetry between matter and antimatter known as CP violation. Physicists have already observed CP violation among particles called quarks, but they know that it isn't pronounced enough to explain why particles of normal matter vastly outnumber particles of antimatter in the universe. Physicists think that if there is CP violation among neutrinos, then it may be more

analogous to the effect that created the matter-antimatter imbalance in the universe.

In fact, researchers in the United States, Japan, and Europe are engaged in experiments in which they use particle accelerators to fire neutrinos hundreds of kilometers through Earth to huge particle detectors. Current efforts seek to pin down, for example,

the neutrinos emanating from the reactors at the Daya Bay Nuclear Power Plant and two neighboring plants in Shenzhen. In making a definitive measurement, they beat out teams working at reactors in France and South Korea and accelerator-based experiments in Japan and the United States.

The measurement of θ_{13} wasn't the only result in particle physics this year. Researchers working with the world's largest atom smasher, the Large Hadron Collider (LHC) in Switzerland, discovered the Higgs boson, the



That was fast! Construction of China's Daya Bay Reactor Neutrino Experiment began in 2007. With 2 months' worth of data, it scooped competitors in Japan, France, Korea, and the United States.

the masses of the neutrinos and not just the differences between them. And scientists in all three regions are planning bigger experiments to search for CP violation among neutrinos. The Daya Bay result gives those efforts an enormous shot in the arm.

The result also marks a coup for Chinese physicists. The Daya Bay team studied

last piece of physicists' standard model. But if LHC researchers do not find new particles beyond those in the standard model, then neutrino physics could be the future of particle physics—as the fact that neutrinos even have mass isn't part of the standard model. If so, the Daya Bay result may mark the moment when the field took off.

GENOMICS BEYOND GENES

A decadelong, \$288 million study reported this year in more than 30 papers showed the human genome to be quite a bustling place, biochemically speaking. The work—called the Encyclopedia of DNA Elements (ENCODE)—builds on the Human Genome Project, which deciphered the order of the bases that are our DNA's building blocks and found that less than 2% of those bases defined genes.

ENCODE researchers took an intensive look not just at genes but at all of the DNA in between. Their results drive home that much of the genome that at one time was dismissed as “junk DNA” actually seems to play an essential role, often by helping to turn genes on or off. They pinpointed hundreds of thousands of landing spots for proteins that influence gene activity, many thousands of stretches of DNA that code for different types of RNA, and lots of places where chemical modifications serve to silence stretches of our chromosomes,

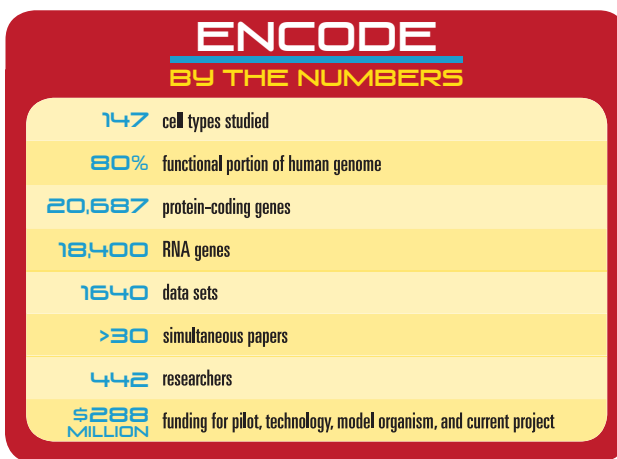
concluding that 80% of the genome was biochemically active. These details provide a much better road map for investigators trying to understand how genes are controlled. Some researchers have already used these insights to clarify genetic risk factors for a variety of diseases, including multiple scler-

osis and Crohn's disease.

When these papers were published in September, the media went wild. ENCODE was hailed in *The New York Times* as a “stun-

home in on different cell compartments, as if they have fixed addresses where they operate, suggesting that they play a role in the cell. Critics argue, however, that it was already known that a lot of RNA was made, and that many of these RNAs may be spurious genome products that serve no purpose. Likewise, one ENCODE researcher found 3.9 million regions across 349 types of cells where proteins called transcription factors bind to the genome—but again, it's unclear how much of that binding is functional.

Nonetheless, ENCODE stands out as an important achievement that should ease the way for more insights into the genome. By combining these data with sampling from another data-intensive effort, the 1000 Genomes Project, researchers discovered that 8% of our DNA appears with little variation throughout the human population—a strong sign that it was important for our evolution. Overall, ENCODE's newly discovered functional regions overlap with 12% of the specific DNA bases linked to higher or lower risks of various diseases, suggesting that the regulation of genes—not just the makeup of the genes themselves—might be at the heart of these risks. Scientists have used this information to home in on relevant genes and cell types in several disorders. Experiments can now unearth the molecular basis of these connections and, from there, identify potential treatments. If that potential is realized, then ENCODE will have earned its accolades as a “stunning resource.”



ning resource” and “a major medical and scientific breakthrough” with enormous and immediate implications for human health. *The Guardian* called it “the most significant shift in scientists’ understanding of the way our DNA operates since the sequencing of the human genome.”

But several scientists in the blogosphere called the coverage overhyped and blamed the journals and ENCODE leaders for overplaying the significance of the results. For example, ENCODE reported that 76% of DNA is transcribed to RNA, most of which does not go on to help make proteins. Various RNAs



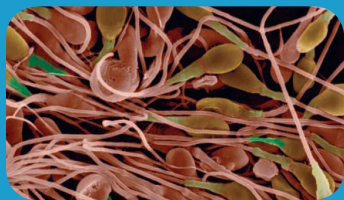
RUNNER-UP

AREAS TO WATCH

ONE CELL AT A TIME

Single-cell DNA sequencing burst onto the scene this year, with advances in microfluidics, the isolation of rare cells, and the ability to decipher these tricky one-shot genomes—milestones that should help break the field wide open in 2013. Even more exciting, some say, are prospects for learning about how cells—particularly brain cells—work by studying the RNA in individual, intact cells.

In the coming year, single-cell sequencing promises to reveal a lot about how cancer cells vary within a tumor and how many copies of genes reside in each cell. Expect continued progress in developing this technology for medical diagnostics for cancer and prenatal applications. Meanwhile, several groups are assessing what genes are doing by measuring in individual cells the messenger RNA that carries their instructions to a cell's protein factories.



PLANCK MAPS THE COSMIC MICROWAVE BACKGROUND

The European Space Agency's Planck satellite will produce the most precise map yet of the afterglow of the big bang, the cosmic microwave background radiation (CMB). The discovery of the CMB in 1965 bolstered the notion that the universe was born in an explosive big bang. Measurements of tiny variations in its temperature in 1992 supported the idea that the universe expanded at greater than light speed in a brief spurt of “inflation.” And the precise mapping of those variations in 2003 helped nail down the composition of the universe: 5% ordinary visible matter, 22% as-yet invisible dark matter, and 73% space-stretching dark energy. Planck will test the now-standard cosmology in greater detail—and could find evidence that the relatively simple scenario isn't quite the whole story.

CONNECTOMES

In 2013, the Human Connectome Project will get into full swing. This \$38.5 million effort, funded by the U.S. National Institutes of Health, aims to scan the brains of 1200 healthy adults, including 300 pairs of twins, to investigate individual variations in the connections between brain regions and how they might account for individual differences in cognition and

SCARY ENGINEERING TAMES MARTIAN TERROR

It looked like a wreck waiting to happen, but the new “sky crane” landing system designed to deliver the massive Curiosity rover safely onto Mars performed flawlessly on 5 August (PDT). Curiosity landed a mere 2.4 kilometers from the center of the bull’s-eye after a 563-million-kilometer journey from Earth—even though engineers had no way to test Curiosity’s “entry, descent, and landing” (EDL) system from beginning to end under martian conditions.

Curiosity mission engineers at NASA’s Jet Propulsion Laboratory in Pasadena, California, pulled off a stunning EDL after thinking far outside the box. Six times before, NASA had landed intact spacecraft on Mars (and once it didn’t work out so well). Three landers came down like 1950s sci-fi spaceships, rockets blazing and landing on legs. The three rovers bounced onto Mars inside NASA’s version of a beach ball. But Curiosity—clamped inside its entry vehicle—weighed in at 3.3 metric tons, too massive for either of the traditional approaches.

So Curiosity engineers considered how their earthly brethren move big things around. Taking their inspiration from cranes and helicopters, they created the sky crane: a platform festooned with retrorockets with the rover, wheels deployed, dangling 7.5 meters below at the end of three cables. The scary-looking contraption could handle a landing mass too large for a beach ball, while safely

setting a massive rover down on inclined slopes that would stymie a legged lander.

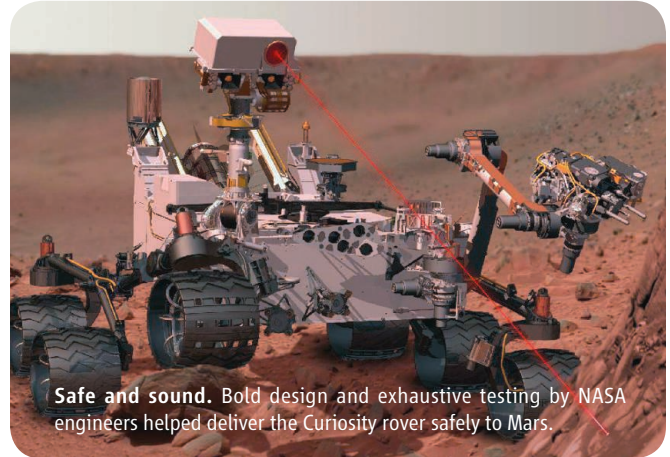
Instruct the platform to cut the cables on touchdown and fly away, and you’ve got a safe landing—in principle.

To make it all work in practice, engineers test, test, and test again. But Curiosity engineers had a problem: Their EDL system—a blazing meteor-

like entry, a parachute descent, and the sky-crane landing, all designed to slow their spacecraft from a hypervelocity speed of 21,240 kilometers per hour to a standstill 7 minutes later—couldn’t be tested on Earth. Earth’s gravity and atmosphere are too different from those of Mars. So they tested components separately as much as they could, for example, by opening the parachute in the world’s largest wind tunnel. Then they tested the system end to end millions of times in a computer. In the end, reality played out as the simulations did—a sign that NASA had taken one more step toward solving the far weightier problem of landing astronauts on Mars.

Engineers got a break when mission planners asked them to have Curiosity touch down up close and personal with the geologically intriguing central mound of Gale crater, a tight spot that no previous mission could have targeted. Instead of streaking in as uncontrolled as a bullet, Curiosity revived a “heritage” concept from the days of the Apollo moon program, when astronaut-bearing capsules guided themselves during reentry into Earth’s atmosphere. Sensing any deviations from its intended flight path, the Curiosity entry vehicle would fire side

RUNNER-UP



Safe and sound. Bold design and exhaustive testing by NASA engineers helped deliver the Curiosity rover safely to Mars.

thrusters to correct its course as it plunged toward the surface. The rover’s spot-on landing reassured planners that NASA can now send a rover to collect samples on Mars and later land a second mission in the same spot to pick up the samples and loft them into Mars orbit for eventual return to Earth.

behavior. Several other projects are zooming in to examine neural connectivity at the cellular level. Advocates and critics have debated how much these maps will advance our understanding of brain function. By this time next year, far more data will help inform the debate.

PIERCING A FRIGID UNDERWORLD

The depths of Antarctica are about to be brought to light. In February, after 14 years of off-and-on drilling through 4 kilometers of East Antarctic ice, Russian scientists stopped just short of the surface of a mysterious subglacial lake likely cut off from the rest of the planet for millions of years. This month, the team returns to Lake Vostok with plans to bring back samples of ice—and, they hope, to discover signs of long-buried indigenous life. U.S.-led and U.K.-led teams are embarking on their own expeditions to study subglacial Antarctic waters. The U.S. team will head to the Whillans Ice Stream, where Antarctic ice joins the Southern Ocean; the U.K. team, to Lake Ellsworth, also on the Western Antarctic Ice Sheet.

CANCER IMMUNOTHERAPY

Recently developed drugs that harness the body’s immune system to fight cancer have beaten back the disease in a small subset of tumor-ridden

patients. Researchers predict that combining two such immunotherapies that target different pathways could pack an even more powerful punch. In 2013, look for early results from clinical trials that pair two antibodies that thwart pathways that tumor cells co-opt to hide from the immune system, and for reports on human studies that combine this brake-lifting strategy with treatments that rev up the body’s immune response.

PLANT POWER

Expect basic plant research to pay off this year, with farmers making use of drought-resistant crops and companies selling the first algae-based diesel fuel. Researchers expect to pin down details of the molecular and genetic components that interact to regulate the growth of plants. Mechanical forces will prove to play a key role in this regulation. Melding genomic, developmental, and ecological studies should help reveal how natural variation can succeed—or fail—to enable plants to adapt to climate change.



FIRST PROTEIN STRUCTURE FROM AN X-RAY LASER

One hundred years ago, physicists showed how x-rays ricocheting through a crystal could reveal the crystal's atomic-scale structure. This year, scientists pushed such "x-ray diffraction" nearly to its ultimate limit when, for the first time, they used an x-ray laser to

stay of structural biology. When many copies of a molecule are arranged in an orderly array called a crystal lattice, they scatter the x-rays from an incoming beam in concert. The pattern of scattering reveals the structure of the crystal, including that of the molecule. Using

circular particle accelerators called synchrotrons to generate x-rays, biologists have determined tens of thousands of protein structures.

Some proteins, such as those found in cell membranes, do not readily form crystals big enough to be studied with synchrotrons, however. So, scientists hope they can probe those tough cases with new x-ray lasers, which are powered by straight-shot

linear accelerators and shine a billion times brighter than synchrotron sources. In

November, researchers

unveiled the first protein structure revealed with such a laser.

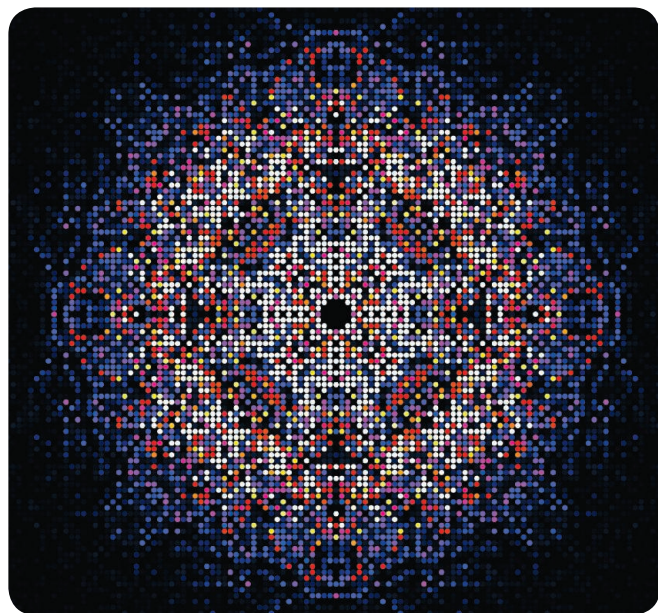
Working with the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory in Menlo Park, California,

researchers from Germany and the United States determined the structure of the inactive "precursor" form of an enzyme that's key for the survival of the single-celled parasite that causes African sleeping sickness, *Trypanosoma brucei*. To produce micrometer-sized crystals of the enzyme, they overexpressed it in cultured cells. They dropped the crystals through the beam of the LCLS, which turned on in 2009. A pulse of x-rays would obliterate a crystal even as it produced a diffraction pattern. Adding up 178,875 individual patterns, researchers determined the precursor's structure, which includes a kind of molecular safety cap that deactivates it. That information could help scientists find a drug to tie up the active form of the enzyme.

With just one new structure in the bag, it's not yet clear that x-ray free-electron lasers (XFELs) will compete with synchrotrons in structural biology. For one thing, researchers were not able to determine the structure of the enzyme de novo from the diffraction data alone, but had to use the known structure of the active enzyme as a starting point. For another, an XFEL serves far fewer users than a synchrotron does. Still, the "diffraction before

destruction" approach takes a qualitative step past what synchrotrons can do. Earlier this year, researchers in Japan turned on their own XFEL, and researchers in Europe are building one that should power up in 2015.

The grand goal is to push x-ray diffraction to its ultimate limit and use an x-ray laser to decipher a protein structure by zapping individual molecules. It's not certain that can be done, but some researchers say the new result suggests that objective may not be too far out of reach.



In sum. Researchers used 178,875 individual laser pulses to generate this diffraction pattern and decipher the structure.

determine the structure of a protein. The advance shows the potential of x-ray lasers to decipher proteins that conventional x-ray sources cannot.

X-ray diffraction has long been the main-



BRAIN-MACHINE INTERFACES START TO GET A GRIP

This week researchers in Pennsylvania reported that a 53-year-old woman paralyzed from the neck down by a genetic neurodegenerative condition had learned to manipulate a robotic arm with her thoughts. Surgeons had implanted two 4×4-millimeter grids of hair-thin electrodes in her brain to capture signals from an area involved in planning hand movements. A computer translated those signals into commands to move the robotic arm, which was engineered to have nearly all the same movement capabilities as the real thing. In videos, the woman uses the arm to grasp and move vari-

ous objects, removing plastic cones stacked on a base and restacking them one by one on another base, for example. The demonstrations represent the most complex movements yet performed by a paralyzed human patient using a brain-machine interface (BMI), as such sophisticated prosthetics are often called.

By demonstrating more fluid and natural movements, this case study improves on another impressive report earlier this year. In that study—the first published demonstration that paralyzed human patients can use a BMI to execute complex movements in three

dimensions—a 58-year-old woman who had been unable to speak or move her limbs for 15 years manipulated a robotic arm with her thoughts, reaching out to grasp a bottle and take a sip of coffee. A tetraplegic man, 66, also learned to touch and grasp objects.

All of this work builds on more than a decade of research with monkeys and other animals. And that work continues to advance. In 2011, researchers described a prosthetic system that provides tactile feedback by stimulating the somatosensory cortex, the brain region responsible for the perception of touch. And in April of this year, a team used signals from electrodes implanted in the motor cortex of the brain to stimulate muscles in the temporarily paralyzed arms of two monkeys, enabling the animals to pick up rubber balls and place them in a chute. Such



MAJORANA FERMIONS, QUASI-HERE AT LAST

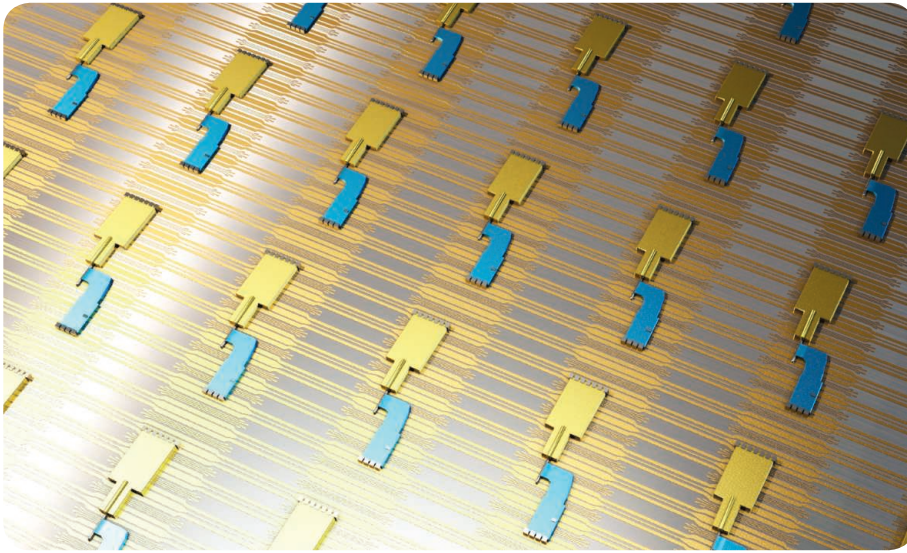
Nanoscience is more than just a fashionable buzzword. It's already paid off in billions of dollars worth of products including better batteries and baseball bats. This year, researchers in the field delivered a different type of value: their first-ever likely particle discovery, known as Majorana fermions.

standing of fermions, particles such as electrons that show a type of angular momentum known as spin, with Albert Einstein's equations of relativity that impact particles traveling near the speed of light. Majorana's insights implied the existence of a new type of fermion that could act as their own anti-

years ago, theorists suggested that the collective motion of electrons in nanoscale wires adjacent to a superconductor may form "quasiparticles" that for all intents and purposes behave as if they were a fundamental Majorana particle themselves. The race was on. This year, a team of physicists and chemists in the Netherlands crossed the line showing compelling evidence that the Majorana quasiparticles exist.

The discovery has already prompted efforts to use the new particles to build a stable quantum computer. Such computers operate on quantum bits, or qubits. Unlike regular bits of digital information represented as 0s and 1s in calculations, qubits can be virtually any combination of a 0 and 1—say, 57% 0 and 43% 1, or 12% 0 and 88% 1. As a result, quantum computers have the potential to store and process information in ways that conventional digital machines can't hope to match. For some types of calculations, crunching just 300 qubits could generate an answer that today's best supercomputers would struggle to solve.

However, current qubit technology is far too fussy for practical computing. The slightest bump in temperature or other outside influence typically wipes out the information stored in a standard qubit. Theoretical calculations show that Majorana fermions should be able to "remember" their quantum state even when buffeted by outside forces. So now the Dutch team and others are hot on the trail to see whether that is the case. If it is, nanoscience may soon be able to add to its bragging rights.



Particle detectors. At the heart of each device in this array is an indium antimonide nanowire, one end is gold-coated and the other is a superconductor (blue). Majorana fermions are produced at the ends of the nanowires.

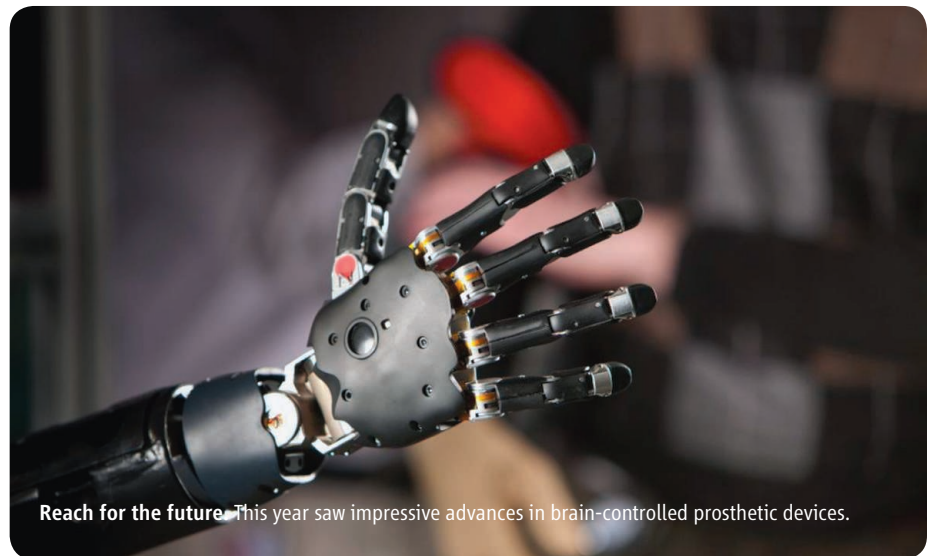
Speculation about the existence of Majorana particles dates back more than 7 decades, when a young Italian physicist named Ettore Majorana crunched some equations in the emerging field of quantum mechanics. His mathematics united the quantum under-

matter and annihilate themselves.

Physicists have long suspected that neutrinos are Majorana fermions. Thus far, they've been unable to nail down the case. And prospects for finding other Majorana fermions long seemed remote. But a few

findings hint at the tantalizing possibility that it may one day be possible to reanimate paralyzed limbs in people.

As hopeful as these developments are, it will be years before large numbers of people can benefit from BMIs. The robotic arms are experimental and extraordinarily expensive, and patients use them only in the lab, aided by a team of technicians. And the movements enabled by BMIs aren't nearly as fast and graceful as the movements made by uninjured individuals. Advances in the algorithms that decode neural signals and convert them into commands a computer or prosthetic limb can understand should help with that. Progress in that area continues apace, but for hundreds of thousands of patients paralyzed by strokes, spinal injuries, and other conditions, it can't come quickly enough.



Reach for the future. This year saw impressive advances in brain-controlled prosthetic devices.

MAKING EGGS FROM STEM CELLS

Researchers have been trying for more than a decade to make egg cells in the laboratory. This year, they took an important step toward that goal, as lab mice gave birth to the first live pups born of eggs derived from mouse embryonic stem (ES) cells. The technique, developed by researchers in Japan,

still requires a mouse to host the developing eggs during a key part of their maturation, so it doesn't achieve the big prize: deriving egg cells entirely in vitro. But it does demonstrate that ES cells can give rise to fertile oocytes, and it gives scientists a way to learn more about how these complex and powerful cells develop.

Egg and sperm cells, also known as germ cells, have a particularly complicated development. They undergo meiosis, a special kind of cell division that leaves them with half the normal number of chromosomes. They also reset the genomic imprinting that helps deter-

mine which genes are turned on and which are turned off. Although pluripotent cells—including ES cells—are capable of becoming any kind of cell in the body, turning them into germ cells in the lab has proved difficult.

In 2011, the same lab in Japan reported that it had turned ES cells into fertile sperm. In 2012, researchers there showed that a similar process can produce eggs. First, they treated the stem cells with a cocktail of growth factors and proteins to form what they call primordial germ cell-like cells, which resemble the precursors of egg and sperm cells found in early embryos. They then mixed the cells with ovarian tissue. The cells formed clusters that resemble miniature ovaries. The scientists

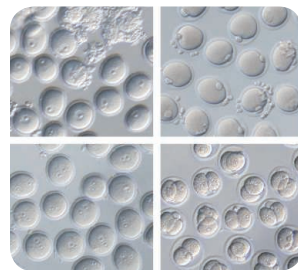
implanted those clusters in the ovaries or kidneys of host mice, and several weeks later they were able to extract mature oocytes.

The scientists used normal mouse sperm to fertilize the oocytes in vitro and then implanted the resulting embryos into foster mothers. The foster mothers gave birth to normal mice, which were then able to go on and have offspring of their own. (The recipe

also works with induced pluripotent stem cells, which are derived from adult cells that have been reprogrammed to behave like embryonic cells.)

The technique doesn't yet work with human cells—and the requirement for ovarian tissue and a live host for part of the development makes it impractical and ethically problematic to try. But having a better way to study the genes and other factors that influence egg cell develop-

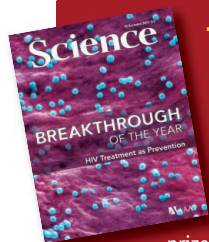
ment could already help researchers understand some kinds of infertility—and could lead to better ways to make these elusive but powerful cells in the lab.



Growing potential. Fertilized lab-derived egg cells yielded embryos—and live mice.

SCORECARD

RATING LAST YEAR'S AREAS TO WATCH



THE HIGGS BOSON

We said that at the rate physicists were collecting data with the world's biggest atom smasher, the Large Hadron Collider, it was "all but a mathematical certainty" that they would either find the long-sought Higgs boson or rule out its existence. It appears that physicists have bagged their prize (see p. 1524). Nature was generous. Math works.



FASTER-THAN-LIGHT NEUTRINOS

As suggested, last year's claim that particles called neutrinos travel faster than light fell apart—but in an unexpectedly spectacular way. Physicists had reported that neutrinos were making the 730-kilometer trip from CERN in Switzerland to the OPERA particle detector in Italy 60 nanoseconds faster than they should at light speed. This February, however, they found that the time discrepancy had been caused by a loose cable connection. In March, two leaders of the 200-member OPERA team stepped down after a vote of no confidence.



STEM-CELL METABOLISM

Scientists made progress this year in understanding the way stem cells use energy and the molecules needed for cell function as they differentiate into various tissues. They uncovered more details about how metabolism influences the reprogramming of mature cells into embryolike ones. It's also increasingly clear that the metabolism of



GENOMIC EPIDEMIOLOGY

This year has shown that whole-genome sequencing of infectious bacteria can help scientists understand and even control disease outbreaks. Researchers used the technique to discover how *Clostridium difficile* spread went on a rampage in hospitals around the world and to track outbreaks of resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* bacteria within a single hospital; in the last two cases, they think their genomic sleuthing may have saved lives.



TREATING INTELLECTUAL DISABILITY

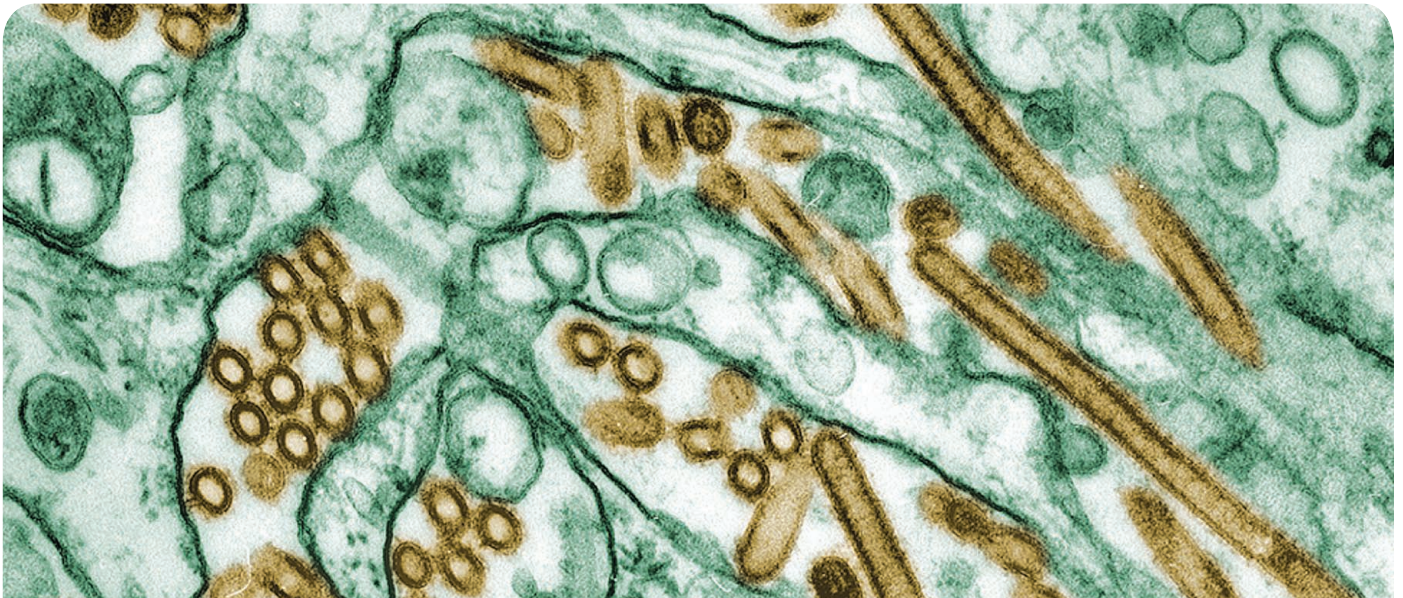
As we predicted, in 2012 animal studies turned up more potential targets for reversing cognitive and behavioral symptoms in autism and related disorders. And clinical trials continued, with researchers reporting encouraging findings with arbaclofen and bumetanide, drugs that enhance certain effects of the neurotransmitter GABA, in people with fragile X syndrome and autism, respectively. Closely watched clinical trials for fragile X with mGluR5 antagonists, which inhibit a receptor for glutamate, another neurotransmitter, should release findings in 2013.



CURIOSITY TO MARS

The Mars Science Laboratory has indeed proved worth watching. Its "7 minutes of terror" descent through the martian atmosphere ended in a safe, spot-on landing (see p. 1529) followed by—so far—months of productive scientific work by the Mini Cooper-sized Curiosity rover. Who could ask for more?





A YEAR ON, THE H5N1 DEBATE REMAINS INFECTIOUS, WITH NO END IN SIGHT

Fiasco. Essential. Inevitable.

Those are just a few of the words that scientists and national security experts have used to describe the global controversy that engulfed influenza researchers this year. The drama began in late 2011 after two science teams showed how to make the H5N1 avian influenza virus—which typically kills birds—transmissible among mammals, potentially opening the door to a deadly human pandemic. Some say the storm—which is still far from over—has exposed long-standing flaws in efforts to prevent dangerous agents from escaping from unsafe laboratories or falling into the hands of terrorists, and highlighted the need for tighter oversight of “dual-use” research that can be used for good and evil. But others fear the episode is fueling a regulatory overreaction that could harm international collaboration and put an end to U.S. funding for potentially valuable science.

There’s one thing that all sides appear to agree on: Nobody wants to repeat the highly publicized meltdown that sowed confusion and contention among scientists, government officials, the media, and the public. “In many ways, it’s been a debacle,” Anthony Fauci, head of the U.S. National Institute of Allergy and Infectious Diseases, told *Science* earlier this year.

Fauci should know. His agency funded the two controversial studies, which raised dual-use concerns after the results were submitted to *Nature* and *Science*. As word leaked out, researchers helped fan the flames when an author of one the studies—virologist Ron Fouchier of Erasmus MC in Rotterdam—told reporters from *Science* and other outlets that his team had engineered a virus that might kill millions. Such suggestions ultimately helped persuade the U.S. National Science Advisory Board for Biosecurity (NSABB), which advises the government on the security risks associated with biological research, to recommend against fully publishing the studies.

That recommendation did little to quell the debate, however, with some scientists calling it misguided while others argued that it didn’t go far enough. *The New York Times* even called on the government to destroy the “doomsday” virus and halt funding for similar research. To help ease fears, in January influenza researchers announced a

voluntary, temporary moratorium on H5N1 experiments that might make the virus more dangerous to humans. And some asked NSABB to reconsider its recommendation. In March, it did—and a majority of the members changed their minds, in part because they learned that Fouchier’s virus was less lethal than originally believed. Some were also encouraged by the release of a new U.S. policy designed to help funders and scientists spot problematic dual-use studies before they begin—potentially heading off future conflicts. With NSABB’s blessing in hand, *Science* and *Nature* finally published the studies.

The end of story, however, isn’t settled. The voluntary moratorium on H5N1 research—which was originally planned to last just 60 days—is still in place with no end in sight. A long-promised follow-up to the March dual-use policy, designed to help U.S. university officials implement the rules, has yet to appear. And this month, U.S. officials introduced a new plot line, unveiling draft guidelines that would bar government funding for H5N1 studies that would enable the virus to gain functions, such as the ability to easily infect humans, which might not naturally evolve. The rules could also require such “gain-of-function” studies to be kept secret.

Not surprisingly, that draft is getting a mixed reception from researchers, with some worrying that it will end U.S. funding for a whole subset of possibly useful studies. Meanwhile, even supporters of such controls say they’ll have only a limited effect if other nations don’t adopt similar rules. “This is a global issue—lots of laboratories can do this type of research, and the U.S. can’t be effective acting alone,” says microbiologist and biosecurity expert Ronald Atlas of the University of Louisville in Kentucky.

The end result: More than a year after the H5N1 controversy erupted, there is still no clear international consensus on which kinds of studies are worth the risk, or how potentially dangerous results should be reviewed or safely communicated to the public and public health experts. Until the confusion clears, experts warn, more messy public battles over finding the right balance between science and security are probably, well, inevitable.

—DAVID MALAKOFF

CREDIT: CDC

THE YEAR IN NEWS

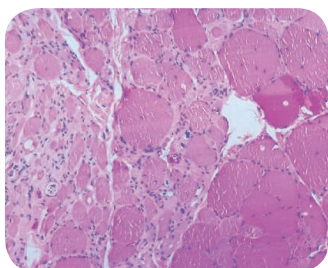
Here are some of the people, places, and events that helped shape the world of science in 2012

JANUARY



Baikonur Cosmodrome, Kazakhstan: Russia's Fobos-Grunt sampling mission fails to escape Earth's orbit on way to martian moon.

Washington, D.C.: Obama administration proposes dismantling Commerce Department and scattering its scientific components across the federal government.



New Delhi: India celebrates going 1 year without a case of polio; no new cases reported in 2012.

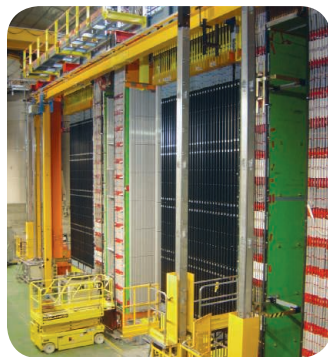
London and Washington, D.C.: Flu researchers announce a 60-day moratorium on risky H5N1 studies in a letter to *Nature* and *Science*. The moratorium remains in effect.

FEBRUARY



Antarctica: A team of Russian scientists finishes drilling 3770 meters through the Antarctic ice to reach the surface of buried Lake Vostok.

Bethesda, Maryland: The National Institutes of Health decides to revise the original design for the National Children's Study after spending nearly \$800 million to plan the monitoring of 100,000 children.



Gran Sasso, Italy: Faulty wiring is found to have caused the anomalous faster-than-light neutrino results announced in September 2011 by scientists in Italy.

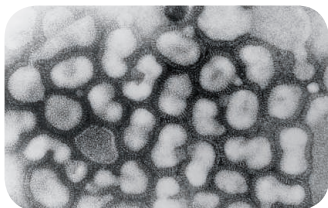
MARCH



Mariana Trench, Pacific Ocean: Filmmaker James Cameron's one-man sub dives to the bottom of Challenger

Deep, the first solo visit to Earth's deepest domain.

Washington, D.C.: U.S. Department of Energy shelves \$1.5 billion Long Baseline Neutrino Experiment, asks Fermilab for cheaper alternatives.



Bethesda: Reversing an earlier decision, the National Science Advisory Board for Biosecurity approves publication of two controversial papers on H5N1's ability to trigger a pandemic.

Washington, D.C.: U.S. government announces rules designed to reduce risk of harmful consequences from experiments using 15 pathogens and toxins.

APRIL



Paris: Contact is lost with Europe's 10-year-old Earth-observing satellite Envisat.



Washington, D.C.: Jim Yong Kim elected as first scientist/physician to lead the World Bank.

MAY



Amsterdam: Australia and South Africa chosen as co-hosts for the \$2 billion Square Kilometre Array.

Mongstad, Norway: Work begins on \$1 billion carbon capture and storage facility, the largest such test site in the world.

Boston: Autopsies of four military veterans find signs of the same neurodegenerative disease found previously in U.S. football players.

JUNE

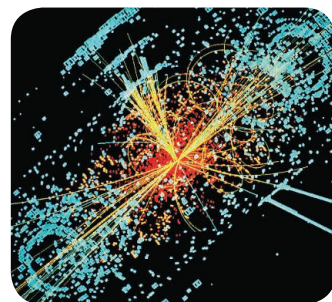


Galapagos Islands: Centenarian Lonesome George, the last giant tortoise in the Galapagos Islands, succumbs to apparent heart failure.



Rio de Janeiro, Brazil: Lack of major commitments dooms Rio+20 conference on sustainable development.

JULY



Meyrin, Switzerland: Physicists at CERN report they've probably found the Higgs boson, the particle that conveys mass to other fundamental particles.

London: U.K. government expects to spend more than \$100 million to subsidize publication in open access journals of research it funds.



Northeastern U.S.: One species of North American bats infected with white-nose syndrome fights the disease by hibernating alone.

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Downloaded from www.sciencemag.org on December 21, 2012

Washington, D.C.: New U.S. law is expected to funnel up to \$20 billion in BP civil fines from the 2010 Gulf oil spill to restoration and research.

Washington, D.C.: Two studies published in *Science* failed to find arsenic in bacterial DNA, refuting controversial work reported in 2010.



Phnom Penh: A mysterious syndrome that killed dozens of children is identified as hand, foot, and mouth disease after patients test positive for Enterovirus 71.

Tokyo: Panel finds that anesthesiologist Yoshitaka Fujii fabricated a record-setting 172 papers.

AUGUST

Bethesda: The National Heart, Lung, and Blood Institute launches massive clinical trial to test whether blocking inflammation can prevent heart disease.



Pasadena, California: NASA's Curiosity rover lands safely on Mars and begins 2-year mission.



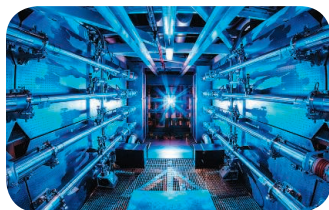
Indianapolis and New York: Long-awaited clinical trial results for bapineuzumab and solanezumab fail to show cognitive benefits for Alzheimer's disease patients.

Kyoto, Japan: Mathematician Shinichi Mochizuki invites colleagues to poke holes in his proof of the abc conjecture.



SEPTEMBER

Hinxton, U.K.: Results from the Encyclopedia of DNA elements (ENCODE) project identifying a high percentage of human DNA with some functionality generate praise and controversy.



Livermore, California: National Ignition Facility fails to meet its own deadline for achieving a self-sustaining fusion reaction.

Saudi Arabia and Qatar: Two cases, one fatal, of a new coronavirus related to SARS triggers worries about a wider outbreak.



Hunan province, China: In a nationwide uproar, critics say that a U.S.-funded study involving genetically modified (GM) golden rice used Chinese children as guinea pigs.



Caen, France: Study claiming that GM maize causes tumors and early death in rats generates headlines—and widespread criticism from food safety agencies.

Alaska: Shell begins exploratory oil drilling in Alaska's Chukchi Sea, the first in more than 2 decades. But technical problems cut the project short.

OCTOBER



L'Aquila, Italy: Six scientists and a government official are found guilty of manslaughter and sentenced to 6 years in prison for making reassuring statements before a deadly April 2009 earthquake.



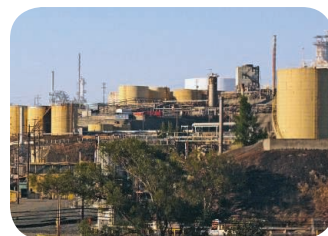
Hyderabad, India: The U.N. Convention on Biological Diversity announces a doubling, to \$10 billion a year, of aid to developing countries by 2015.

Cambridge, U.K.: First articles appear in *eLife*, a new open access journal backed by the Howard Hughes Medical Institute, the Wellcome Trust, and the Max Planck Society.



Austin: Nobel laureate Alfred Gilman resigns from \$3 billion Cancer Prevention and Research Institute of Texas along with dozens of peer reviewers to protest agency's peer review practices.

NOVEMBER



Sacramento: California's cap-and-trade program, the broadest in the nation, began auctioning permits to businesses in an effort to regulate release of greenhouse gases.

Washington, D.C.: BP to pay \$2.5 billion for research and restoration efforts as part of guilty plea in criminal case from oil spill.

Brussels: The European Commission's approval to market Glybera to treat a rare disease called lipoprotein lipase deficiency makes it the first gene therapy drug in the Western world.

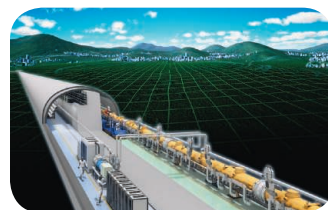


Tilburg, the Netherlands: Investigators say social psychologist Diederik Stapel has committed fraud in at least

55 of his 137 papers.

Cape Town: Disappointing results from first phase III trial of a malaria vaccine dim prospects for RTS,S vaccine.

DECEMBER



Tokyo: Scientists complete technical design for proposed International Linear Collider, a \$10 billion facility that Japan hopes to host.

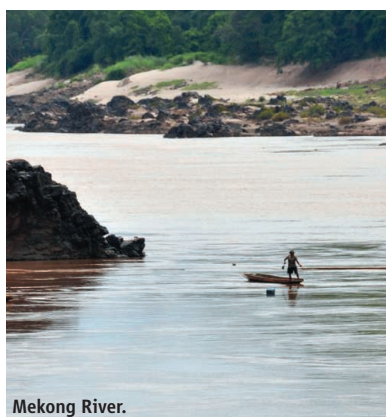
Brussels: E.U. officials endorse a unified patent system that they hope will take effect in 25 countries in 2014.

LETTERS

edited by Jennifer Sills

Dam Threatens Mekong Ecology

THE NEWS OF THE WEEK STORY "MEGADAM GETS GREEN LIGHT" (9 November, p. 726) seems to provide strong support for the cautionary Letter by B. Gong *et al.* ("Limits to religious conservation efforts," 9 November, p. 740) arguing that while Buddhism may be a



Mekong River.

powerful resource for conservation, it cannot replace strong environmental governance and policy. The Xayaburi Dam is a very bad idea and clearly represents a massive threat to the ecology of the Mekong River and its people. Yet it proceeds with the support of at least two predominantly Buddhist countries, Laos and Thailand, where protests by Buddhist citizens have gone unheeded

(1). The environmental governance and policy offered by Laos and Thailand to support the dam is especially dangerous in that it sets a bad precedent that will most certainly affect the construction of at least 11 additional dams planned for the Mekong River (2). The decision to build the dam is based on a seriously flawed environmental impact assessment (3) and totally ignores an earlier agreement in 2011 (4) with Cambodia, Thailand, and Vietnam that would extend the decision-making process until major gaps in the current knowledge about the environmental and social impacts of the Xayaburi Dam are reconciled. When those knowledge gaps are filled with factual information, it will be abundantly clear that the Xayaburi dam should not be built.

GUY R. LANZA

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References

1. "Thai government supports Xayaburi Dam," *Bangkok Post*, 11 June 2012.
2. M. Osborne, *Asia Pacific J.* (11 January 2010); www.japanfocus.org/-Milton-OSborne/3286.
3. G. R. Lanza, "Review of the Ch. Karnchang public company limited environmental impact assessment (EIA) report to the Mekong River Commission" (International Rivers, Berkeley, CA, 2011); www.internationalrivers.org/files/attached-files/lanza_water_quality_final.pdf.
4. Mekong River Commission, "Lower Mekong countries take prior consultation on Xayaburi project to ministerial level" (2011); www.mrcmekong.org/news-and-events/news/lower-mekong-countries-take-prior-consultation-on-xayaburi-project-to-ministerial-level/.

Mobilizing Religion and Conservation in Asia

IN THEIR LETTER "LIMITS TO RELIGIOUS CONSERVATION EFFORTS" (9 November, p. 740), B. Gong *et al.* caution conservation practitioners that there are limits to the effect that Buddhist influence alone can have in reducing environmental degradation caused by economic development. They cite prayer animal release—a practice in which animals are trapped, sold, and then released into the wild as a form of prayer—as evidence of Buddhists' lack of understanding of ecology.

Religious leaders, government, and the local and international conservation community are currently addressing the unsustainable Buddhist practice of releasing animals. A recent policy paper by the Religion and Conservation Research Collaborative (RCRC), a committee of the Religion and Conservation Biology Working Group of the

Society for Conservation Biology, in July 2012, stated that Buddhists are acting in good faith and should be provided with alternatives that could achieve the compassionate spirit of prayer animal release in an ecologically responsible way (1).

As an offshoot of RCRC policy, an e-mail forum involving more than 40 scientists and scholars from around the world (the Mercy Release Discourse, 13 to 17th August 2012) identified a few ideas: (i) Encourage Buddhist practitioners to adopt a domestic animal (e.g., a cow) destined for the slaughter and care for it until it dies naturally or to sponsor accredited farm animal sanctuaries. (ii) Encourage Buddhist practitioners to support conservation programs of endangered species. (iii) Facilitate support for Buddhist practitioners who want to rehabilitate animals that are sick or injured, reintroduce wildlife into the wild, or send wildlife to rescue centers.

Some Buddhist groups in China and elsewhere in the region have already put these

alternatives into action (2). In Singapore, religious adherents have been attempting to address the concerns as well (3). Although imperfect, these developments suggest the potential for conservation inspired by religion and for science to involve religion in collaboration and dialogue, rather than simply criticism.

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References

1. S. M. Awoyemi *et al.*, Society for Conservation Biology, "Religion and Conservation Research Collaborative (RCRC) of the Religion and Conservation Biology Working Group (RCBWG) Society for Conservation Biology (SCB)

Position on the Religious Practice of Releasing Captive Wildlife for Merit" (2012); www.conbio.org/policy/religion-and-conservation-biology-working-group-policy-position-on-the-rele.

2. Environment and Animal Society of Taiwan, Humane Society International, American Buddhist Confederation, Taiwan's Department of Agriculture, "International Symposium on Buddhist Animal Release and Protecting Life, September 25–26, Taipei, Taiwan" (2012) [in Chinese]; www.forest.gov.tw/public/Attachment/29281045671.pdf.
3. K. Y. Chong, "Animal Release: Science, Compassion and Religion," *Wild Singapore* (2011); <http://wildsingaporenews.blogspot.com/2011/05/animal-release-science-compassion-and.html>.

Shark Sanctuaries: Substance or Spin?

AS SHARK POPULATIONS COLLAPSE AND PUBLIC concern rises, some national governments have established shark sanctuaries. These countries, such as Marshall Islands, Maldives, and Venezuela, have been touted to be "safeguarding" (1) and "protecting" (2) sharks. The Marshall Islands sanctuary was hailed as the "strongest legislation to protect sharks we have seen" (3). Fiji bucked the trend recently by deciding not to declare their national waters a sanctuary, thereby attracting press attention and criticism (4). This raises



the question: What are shark sanctuaries, and does their creation result in effective shark conservation and management?

Given that studies show shark populations are declining mainly as a result of overfishing (5, 6), no-take marine zones might seem like a logical and effective way to curb mortality and boost populations. However, what constitutes a sanctuary varies among countries, and often is not synonymous with no-take zones. For example, the Marshall Islands bans commercial fishing yet allows small-scale fishing of sharks (7). The Maldives has banned commercial fishing only in waters out to 12 nauti-

cal miles (8), and Venezuela has banned commercial shark fishing in less than 1% of their waters (9).

Even with sufficiently protective bans, shark sanctuary creation is only the first step; the real challenge is ensuring effectiveness through strict monitoring and enforcement (10, 11), which requires sustainable financing. Indeed, Fiji's offshore fisheries officer stressed difficulties with monitoring and enforcing a total ban on shark fishing (12). Alternatively, allocating capacity toward scientific data collection would allow experts to evaluate effectiveness of management measures and inform long-term regional and global population assessments.

Shark sanctuaries provide hope, but there is no scientific evidence that they are effective—yet. Even worse, the positive press attention surrounding shark sanctuaries may preclude more effective conservation management. Sanctuaries should not substitute for rigorous, science-based management.

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References

1. K. Escobar, "Leaders launch new shark conservation effort," The Pew Charitable Trusts (2011); www.pewenvironment.org/news-room/press-releases/leaders-launch-new-shark-conservation-effort-85899364288.
2. R. Brittin, "Venezuela ends shark finning, creates protected area," The Pew Charitable Trusts (2012); www.pewenvironment.org/news-room/press-releases/venezuela-ends-shark-finning-creates-protected-area-85899376580.
3. D. Braun, "Marshall Islands declare world's largest shark sanctuary," *National Geographic* (3 October 2011).
4. T. Tokalau, "No shark sanctuary in Fiji," *The Fiji Times* (13 August 2012).
5. N. K. Dulvy *et al.*, *Aquat. Conserve. Mar. Freshwater Ecosyst.* **18**, 459 (2008).
6. J. D. Stevens, R. Bonfil, N. K. Dulvy, P. A. Walker, *ICES J. Mar. Sci.* **57**, 476 (2000).
7. J. Appelbaum, "Republic of the Marshall Islands shark sanctuary," Marshall Islands Marine Resources Authority (2011).
8. Republic of Maldives, Ministry of Fisheries and Agriculture,

"Maldives imposes shark hunting ban," The President's Office (2009).

9. D. Main, "Venezuela shark finning ban announced as country establishes sanctuary," *The Huffington Post* (22 June 2012).
10. W. D. Robbins, M. Hisano, S. R. Connolly, J. H. Choat, *Curr. Biol.* **16**, 2314 (2006).
11. M. R. Heupel *et al.*, *Fish. Res.* **95**, 350 (2009).
12. E. Turagaiviu, "Shark sanctuary not good for Fiji," *Fiji News* (13 August 2012).

CORRECTIONS AND CLARIFICATIONS

AAAS News & Notes: "AAAS members elected as fellows" (30 November, p. 1168). The following lines contained errors. The correct information follows: Keivan Guadalupe Stassun, Vanderbilt Univ.; S. Lawrence Zipursky, Univ. of California, Los Angeles; Alcino J. Silva, Univ. of California, Los Angeles; Gene D. Sprouse, Stony Brook Univ. The HTML and PDF versions online have been corrected.

Research Articles: "A reconciled estimate of ice-sheet mass balance," by A. Shepherd *et al.* (30 November, p. 1183). The estimated individual rates of ice loss for Greenland and Antarctica did not always sum to the estimated rates of loss for the ice sheets combined because the authors calculated the combined trends after adding the time series. The revised rate of ice-sheet mass balance is -142 ± 49 for the Greenland Ice Sheet from 1992–2011 and -211 ± 37 from 2000–2011. The revised rates of ice-sheet loss are 1350 ± 1010 and 2700 ± 930 for Antarctic and Greenland, respectively. Additionally, ref. 48 should have appeared as follows: "48. H. J. Zwally *et al.*, *J. Glaciol.* **57**, 88 (2010)." These changes have been made to the HTML and PDF online versions of the paper.

TECHNICAL COMMENT ABSTRACTS

Comment on "The Local Structure of Amorphous Silicon"

Sjoerd Roorda and Laurent J. Lewis

Treacy and Borisenko (Reports, 24 February 2012, p. 950) argue from reverse Monte Carlo modeling of electron diffraction and fluctuation electron microscopy data that amorphous silicon is paracrystalline and not described by a continuous random network. However, their models disagree with high-resolution x-ray measurements and other evidence, whereas the agreement with fluctuation electron microscopy is at best qualitative.

Full text at <http://dx.doi.org/10.1126/science.1221738>

Response to Comment on "The Local Structure of Amorphous Silicon"

M. M. J. Treacy and K. B. Borisenko

The averaged diffraction data alone cannot distinguish between models with different heterogeneous structures at length scales of about 2 nanometers, even when using high-resolution data. Although our approach to calculating diffraction intensities from the model differs from that of Roorda and Lewis, paracrystallinity in amorphous silicon is undeniably evident in the raw experimental fluctuation electron microscopy data.

Full text at <http://dx.doi.org/10.1126/science.1222571>

Letters to the Editor

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Comment on “The Local Structure of Amorphous Silicon”

Sjoerd Roorda and Laurent J. Lewis

Treacy and Borisenko (Reports, 24 February 2012, p. 950) argue from reverse Monte Carlo modeling of electron diffraction and fluctuation electron microscopy data that amorphous silicon is paracrystalline and not described by a continuous random network. However, their models disagree with high-resolution x-ray measurements and other evidence, whereas the agreement with fluctuation electron microscopy is at best qualitative.

A Report by Treacy and Borisenko (*1*) on reverse Monte Carlo modeling of the reduced radial distribution function $G(r)$ (RDF) and fluctuation electron microscopy (FEM) of amorphous silicon (a-Si) claims that a-Si is inhomogeneous on the 1- to 2-nm length scale, consistent with a fully paracrystalline (PC) model; the authors conclude that the continuous random network (CRN) model must be dismissed and that a-Si consists of paracrystallites embedded in a medium considerably more disordered than a CRN. This would be a far-reaching conclusion. However, the simulations do not provide quantitative agreement with the FEM data and disagree with high-resolution x-ray data of pure a-Si. Further, a true CRN, with or without voids, has not been included in the modeling and therefore cannot be excluded.

The agreement between model and experimental data shown in Fig. 1B in (*1*) is qualitative only. The experimental FEM signal was multiplied by a factor $\gamma = 20$, attributed to decoherence due to inelastic scattering and insufficient knowledge of the sample thickness (*2*). However, the same authors in another paper explain that γ , which varies between 10 and 48, is not fully understood and that “results should not be interpreted in absolute terms” (*3*). Moreover, the FEM intensity measured on the same sample at different microscopes can vary by a factor of 20 (*4*). According to table 1 in (*1*), there are 94 “paracrystalline” atoms out of 1728 (i.e., about 5%). The ordering of the remaining 1634 atoms is not discussed in detail but is stated to be neither topologically crystalline nor that of a true CRN because of ring statistics. Thermal annealing is said to reduce the FEM by a factor of only 2, but other reports have observed a reduction by a factor of 5 (*5*) to 10 (*4, 6*). If one takes at face value the absolute intensities of measured and modeled FEM data, then the amount of paracrystallite matter in well-annealed a-Si may be as low as 1/20 of 1/10 of 5% (i.e., 300 parts per million).

Treacy and Borisenko (*1*) provide no evidence for excluding voids in an otherwise fully connected CRN structure giving rise to the FEM. This is a real possibility (*7*), which refutes the notion that paracrystallites are the only possible explanation of the observed FEM. The authors speculate that paracrystals nucleate at void surfaces but present no evidence and do not explain why these must be introduced when voids can account for the FEM signal (*7*). In fact, neither do they exclude the CRN model: The simulations start from random configurations that are not true CRNs (for example, the initial configuration is not tetrahedrally bonded, and the final configuration has a high proportion of three-membered rings and does not fit the RDF). The CRN model, with or without voids, has not been included in the reverse Monte Carlo modeling and therefore cannot be dismissed.

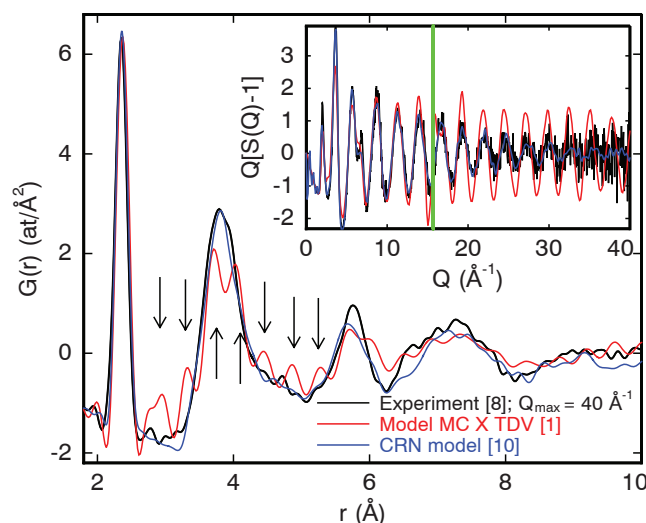
For the paracrystallite model to be valid, the RDF of pure a-Si must be accounted for. Figure 1A of (*1*) presents low-resolution data only. In Fig. 1, we compare the experimentally determined $G(r)$ at high resolution (*8*)—here, the maximum scattering vector $Q_{\max} = 40 \text{ \AA}^{-1}$ and a slight damping has been applied—with the $G(r)$ for model MC X TDV [calculated directly from the atomic coordinates provided in the supporting online material for (*1*)],

smoothed so as to show comparable widths of the first coordination peak. Clearly, the model deviates significantly from pure a-Si; arrows indicate positions of sharp peaks in the model that do not correspond to sharp features in the measured $G(r)$. Only the peak at $4.45 \text{ \AA}$ corresponds to a feature visible in high-resolution RDFs (*9*), but it appears upon annealing, whereas the FEM intensity is known to reduce upon annealing (*4–6*). The first shell coordination number of the paracrystallite models (~ 3.6) is significantly less than the measured value (3.88 ± 0.01 in annealed a-Si), and the width of the distribution of tetrahedral angles of the models (16°) (*3*) is nearly twice the measured value ($9.63^\circ \pm 0.08^\circ$). These deviations are so significant as to invalidate the claim that the models provide a correct fit to the a-Si RDF. In contrast, the blue curve, corresponding to a 1000-atom CRN model (*10*), fits the experimental $G(r)$ much better. Similar conclusions are drawn when comparing the data and models in reciprocal space, as depicted in the inset, which shows the corresponding interference functions. The MC-X-TDV model agrees with the data up to $Q = 15 \text{ \AA}^{-1}$, but for larger values it does not exhibit the strong damping shown by both the experimental data and the CRN model. Such a strong disagreement was not found for CRN models containing voids, which also succeeded in explaining the FEM data (*7*).

Other evidence should be taken into account. The absence of a large small-angle x-ray scattering signal (*11*) argues against a mixed phase (PC-disordered) structure, and so do the observations from impurity segregation (*12*) and crystallization through nucleation and growth (*13*), demonstrating that a first-order phase transition separates a-Si from c-Si. A mixed-phase model for a-Si, such as PC, is fundamentally incompatible with these observations.

Thermal annealing of a-Si induces structural relaxation, and many of its aspects (tetrahedral and dihedral bond ordering, defect removal, and changes in the vibrational spectrum) can and have been understood in terms of the relaxation of defected

Fig. 1. Black curve: RDF from pure a-Si [after (*8*)]. Red curve: RDF for model MC X TDV, according to atomic coordinates provided with (*1*) and smoothed to the same first-peak width. Blue curve: RDF for a 1000-atom CRN model (*10*). Arrows indicate peaks in the model RDF that do not correspond to features in the experimental data or the CRN. The inset shows the corresponding interference functions; the vertical line near $Q = 15 \text{ \AA}^{-1}$ is the upper limit of the diffraction data considered in the reverse Monte Carlo modeling.



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CRN models. If the FEM from as-prepared a-Si were due to small crystallites embedded in a CRN (because of incomplete transformation to the amorphous phase), and if one views these crystallites as prenucleation subcritical (and therefore unstable) embryos (14), then one would expect the FEM amplitude to decrease upon annealing because most of those nuclei would dissolve. This is exactly what is observed (4–6), and it indicates that in well-annealed a-Si the volume fraction of paracrystalline matter is insignificant.

It is entirely true that the ideal, fully connected, four-fold coordinated CRN is never realized. It is equally true that the perfect crystal

does not exist, except in our imagination. Real crystals are best viewed as ideal crystals with defects and imperfections. Likewise, a-Si is best viewed as a CRN with imperfections—for example, vacancies or voids. High-resolution x-ray measurements support this view fully.

References

1. M. M. J. Treacy, K. B. Borisenko, *Science* **335**, 950 (2012).
2. M. M. J. Treacy, J. M. Gibson, L. Fan, D. J. Paterson, I. McNulty, *Rep. Prog. Phys.* **68**, 2899 (2005).
3. K. B. Borisenko *et al.*, *Acta Mater.* **60**, 359 (2012).
4. B. Haberl, thesis, Australian National University, Canberra (2010).
5. B. Haberl *et al.*, *J. Appl. Phys.* **110**, 096104 (2011).
6. J.-Y. Cheng, J. M. Gibson, D. C. Jacobson, *J. Mater. Res.* **16**, 3030 (2001).
7. P. Biswas, R. Atta-Fynn, S. Chakraborty, D. A. Drabold, *J. Phys. Condens. Matter* **19**, 455202 (2007).
8. K. Laaziri *et al.*, *Phys. Rev. B* **60**, 13520 (1999).
9. S. Roorda *et al.*, *Phys. Rev. Lett.* **108**, 255501 (2012).
10. G. T. Barkema, N. Mousseau, *Phys. Rev. B* **62**, 4985 (2000).
11. D. L. Williamson *et al.*, *Appl. Phys. Lett.* **67**, 226 (1995).
12. D. C. Jacobson, J. M. Poate, G. L. Olson, *Appl. Phys. Lett.* **48**, 118 (1986).
13. U. Köster, *Phys. Status Solidi* **48**, 313 (1978).
14. K. F. Kelton, A. L. Greer, C. V. Thompson, *J. Chem. Phys.* **79**, 6261 (1983).

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Response to Comment on “The Local Structure of Amorphous Silicon”

M. M. J. Treacy^{1*} and K. B. Borisenko^{2,3}

The averaged diffraction data alone cannot distinguish between models with different heterogeneous structures at length scales of about 2 nanometers, even when using high-resolution data. Although our approach to calculating diffraction intensities from the model differs from that of Roorda and Lewis, paracrystallinity in amorphous silicon is undeniably evident in the raw experimental fluctuation electron microscopy data.

The discrepancy in the reduced radial distribution function (RDF) plots arises because Roorda and Lewis (1) are probably calculating the RDF directly from our model atomic coordinates, which are periodic (2). Our experimentally constrained structural relaxation (ECSR) method is driven by diffraction information, which is not periodic. We match diffraction data to that computed from an isolated unit cell and ignore the periodicity of the model. There are pros and cons for each method for comparing to diffraction data. Our choice was motivated by a desire to preserve the valuable diffraction information that guides our structural relaxation procedure. For larger models, the two methods should converge to the same RDF.

We agree that higher-resolution diffraction data provides additional information and must therefore improve the structural accuracy. However, the RDF is a sample average and cannot tell us much about structural heterogeneity in amorphous samples, particularly in the diffraction-amorphous regime at structure-correlation length scales of about 2 nm. Diffraction data are collected over a large sample volume and so are homogeneously averaged. The data are then processed isotropically to produce a smoothed radial average, the RDF, which obviously cannot be correct for any individual atom in the disordered sample. A high-resolution RDF can inform us only about the quality of a model measured as a homogeneous average, and other topologically distinct models can also fit this averaged data. The central issue is to find the topologically correct model. The fact that a periodic CRN model matches the homogeneously averaged data does not mean that it is therefore the only structural model that can match that average. We have shown that both random and CRN models match the experimental RDF but produce flat calculated fluctuation electron microscopy (FEM) intensity variance, contrary to

experiment. FEM diffraction intensity variance data provides additional information that shows that the homogeneous hypothesis (the CRN model) cannot be correct for a-Si.

To explore the claim that higher-resolution diffraction data will resolve the structure better at the 2-nm length scale, we computed a high-resolution

diffraction pattern (angular wave vector $Q_{\max} = 40 \text{ \AA}^{-1}$, using our nonperiodic diffraction method) from a CRN model, shown in projection in Fig. 1A. Using this model's computed diffraction pattern as noise-free input data, along with the experimental FEM variance data, we applied the ECSR procedure to obtain the model shown in projection in Fig. 1B, which stubbornly exhibits paracrystalline order. The original CRN model is not recovered, even though it was the CRN's high-resolution diffraction data that was used. The FEM data favor the paracrystallite model over the CRN. The reduced diffraction intensity plots for the two models (Fig. 1C) are essentially identical, overlapping almost perfectly, illustrating that averaged diffraction alone cannot tell the two models apart.

Consequently, Roorda and Lewis overreach with their claim that “High-resolution x-ray measurements support [the defective CRN model] fully.” The strongest statement that they are entitled to make about their measurements is that their experimental RDF is not inconsistent with their particular CRN model. The good agreement

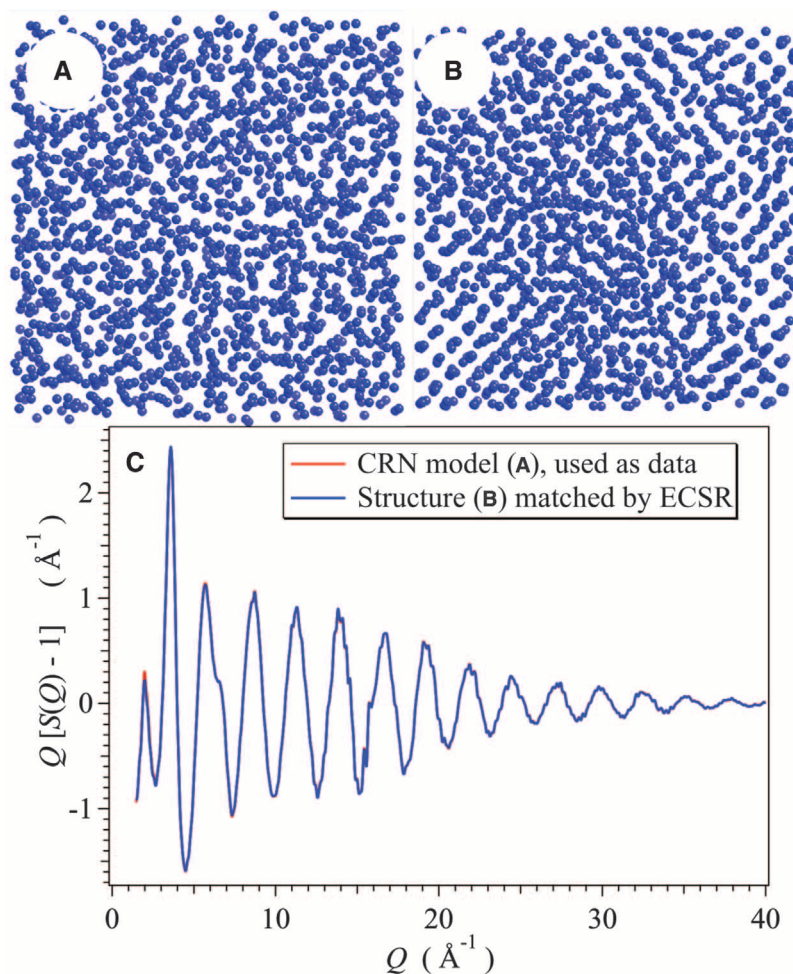


Fig. 1. (A) Projection through the CRN model that was used to generate a high-resolution “target” diffraction pattern. (B) The model found that fit both the CRN target diffraction data and the intensity variance data best. (C) The models have identical reduced diffraction intensities. Diffraction alone cannot differentiate the two models.

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between averaged diffraction data alone does not prove that the CRN is the only possible model.

Roorda and Lewis estimate that the amount of paracrystalline material may be as low as 300 parts per million. This would correspond to half an atom in our 1728-atom models. Their estimate is too low. We have employed a stringent definition of a paracrystallite, which involves topological constraints out to the third-nearest neighbor to define a local cluster of 29 atoms (3, 4). By one measure, we can say that this counts only as one paracrystalline atom, despite the entourage of 28 other atoms that help define it.

Roorda and Lewis remind us that models containing voids can also explain the FEM data (5). This translates into the extraordinary claim that voids provide a better explanation for the presence of crystalline 111, 220, and 311 intensity-variance peaks than do the topologically cubic-structure paracrystallites. In principle, void models are a legitimate part of the solution space that the ECSR method explores, and yet voids do not emerge as viable structures relative to paracrystallites. We have explored models that are seeded with voids at the start of ECSR processing, because there is the possibility that the reduced constraints at the internal void surfaces might encourage nucleation of paracrystallites. This nucleation did not happen in our ECSR runs. Nevertheless, despite the absence of paracrystallites, the final models matched both the diffraction and the variance data, although the Tersoff energy was high relative to typical paracrystallite models. The void-seeded models contained implausible medium-range correlations where {111}-type density modulations appeared in projection when the models were viewed at certain angles, but the {111} short-range structural correlations needed to construct crystallographically true {111} planes were absent. An example of this phenomenon, where medium-range order has little or no accompanying short-range order, is shown in (6). In any event, Roorda

and Lewis state that small-angle x-ray scattering data argue against a mixed-phase structure. Shouldn't this also rule out the void model? However, it is not so clear that this necessarily rules out the paracrystallite model, because the density fluctuations (silicon against silicon) are much more subtle.

Contrary to Roorda and Lewis's claim that paracrystallites dissolve with increasing temperature, which is pointed out as an inconsistency with the physical expectation of crystallization, an earlier FEM study (7) on the a-Si to c-Si phase transition shows that there is a continuous evolution of an ordered Si phase as temperature increases. Diffraction data misleadingly show a "sudden" onset of crystallinity out of the diffraction-amorphous state much later than the actual onset of crystallization, as observed by FEM data. It cannot be overemphasized that homogeneous diffraction alone is insensitive to structural inhomogeneity smaller than about 2 nm in extent.

Roorda and Lewis declare that the FEM method "is at best qualitative." Presumably, their view is that the RDF method is quantitative. A more valuable measure of these techniques is the information they provide about the sample. The RDF method, being an isotropic average of a two-body data set, tells us very little quantitatively about the sample—the average nearest neighbor distance, the average coordination number, and the mean density—and it determines these values precisely. Conversely, the FEM variance data are a complex four-body data set that is rich with details about spatial heterogeneity but is difficult to invert into a specific structure. Just as the mean tells us nothing about the variance, the variance tells us nothing about the mean. It is true that not all of the contributions to the FEM signal are understood fully—in particular, the decoherence effects that strongly suppress the diffraction speckle that FEM examines. It is not unreasonable to use hidden-parameter inferencing to refine the γ

factor, which accounts phenomenologically for decoherence, along with the structural parameters. The γ factor is linearly independent of the other parameters. It should not be overlooked that the raw FEM data show strong evidence for {111}, {220}, and {311} paracrystalline correlations before any numerical processing is done. The raw data alone are inconsistent with a CRN model.

Fluctuation EM, and the ECSR method for inverting the data, are still emerging techniques that can be improved. With modern-day scanning transmission electron microscopes, FEM is a straightforward technique to implement. The ECSR method is computationally intensive and can clearly be improved to work with bigger models. We maintain that the paracrystallite model for a-Si is broadly correct and supersedes the interrupted-CRN model favored by Roorda and Lewis, even for annealed material. More does need to be done to narrow further the set of paracrystalline macrostates that match the data. Although this is an old subject, there is still plenty of scope for further advances in our understanding of the structural details of amorphous silicon.

References and Notes

1. S. Roorda, L. J. Lewis, *Science* **338**, 1539-b (2012); www.sciencemag.org/cgi/content/full/338/6114/1539-c.
2. M. M. J. Treacy, K. B. Borisenko, *Science* **335**, 950 (2012).
3. C. S. Mariani, L. W. Hobbs, *J. Non-Cryst. Solids* **119**, 269 (1990).
4. M. M. J. Treacy, P. M. Voyles, J. M. Gibson, *J. Non-Cryst. Solids* **266**, 150 (2000).
5. P. Biswas, R. Atta-Fynn, S. Chakraborty, D. A. Drabold, *J. Phys. Condens. Matter* **19**, 455202 (2007).
6. K. B. Borisenko *et al.*, *Acta Mater.* **60**, 359 (2012).
7. P. M. Voyles, J. E. Gerbi, M. M. J. Treacy, J. M. Gibson, J. R. Abelson, *Phys. Rev. Lett.* **86**, 5514 (2001).

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HISTORY OF SCIENCE

Of Chymists and Kings

Anthony Grafton

Early in the 1940s, John Maynard Keynes composed an ambivalent tribute to Isaac Newton (*1*). Since the Enlightenment, Newton had been remembered “as the first and greatest of the modern age of scientists, a rationalist, one who taught us to think on the lines of cold and untinctured reason.” But Newton’s long-suppressed manuscripts—many of which Keynes himself bought at auction and presented to King’s College Cambridge—told a different story. The real Newton was an “unbridled addict” of alchemy. He had tried for decades to “read the riddle of tradition, to find meaning in cryptic verses, to imitate the alleged but largely imaginary experiments of the initiates of past centuries.” Keynes described the thousands of pages Newton had devoted to this pursuit as “wholly magical and wholly devoid of scientific value.”

Over the past half-century, historians of alchemy—especially William Newman and Lawrence Principe—have taught us to see the papers that dismayed Keynes in a very different light. The alchemy Newton practiced was a genuine scientific tradition, which took shape over centuries and involved both hands-on experimentation and sophisticated theories.

In *The Secrets of Alchemy*, Principe has pulled together the threads of his research and that of many colleagues. This elegant, readable book, packed with information and revelation, covers the history of alchemy from its shadowy origins in Hellenistic Egypt to its scholarly recovery in the 20th century. Principe traces the contours of a millennial tradition and shows exactly why Newton and many other brilliantly gifted scientists found so much promise in it.

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The Secrets of Alchemy

by Lawrence M. Principe

University of Chicago Press, Chicago, 2013. 295 pp. \$25, £16. ISBN 9780226682952.

The story is not simple: nothing about alchemy is. Alchemists often presented their findings in a deliberately obscure way. They used pseudonyms, forged supposed ancient authorities, and identified vital ingredients with Decknamen (code names), often based on a metaphorical connection to the actual substances to be used. And they pursued many goals, small and large. The search to transmute base metal into gold was only one thread in an immense and varied tapestry.

More important, alchemists often disagreed about basic points: for example, whether substances produced by alchemy could ever match those produced by nature. Avicenna insisted that they could not; Roger Bacon argued, just as strongly, that they could. Medicine has served as both a model for alchemy, seen as therapy for metals, and an application for it, especially thanks to its vociferous Renaissance practitioner Paracelsus.

Early alchemical texts are likely to baffle the modern reader who confronts them for the first time. Basic elements of the art—like the numerological systems used by early alchemists to grade the transformative powers of particular substances—seem arbitrary, if one

does not realize that many saw numbers and their ratios as keys to a deeper reality. Many alchemists explicitly stated that their discoveries came not only from direct, dirty-handed manipulation of metals and acids but also from divine revelations, which they received, and reported, in the form of complex visions.

For all the air of mystery, Principe shows, alchemy has always had a strong hands-on component. Alchemical practitioners interacted with makers of coins, miners, dyers, and others who knew materials and their powers directly. Even the most abstract or mythical-sounding texts are often enciphered recipes. Watching Principe decode a set of images and words and then carry out the manipulations they call for is as exciting as watching a skilled magician carry out his best tricks.

Basil Valentine—the pseudonym for a group of Renaissance alchemical writers—instructs the reader: “the king’s crown should be pure gold, and a chaste bride should be married to him. Take the ravenous grey wolf ... [that] by birth is a child of old Saturn.... Throw the king’s body before him.” It sounds (and the associated image looks) completely bizarre. To Principe, though, the “riddle is relatively easy.” He identifies the king as gold, the king of the metals. Saturn is lead, so his child should be something related to lead: antimony ore or stibnite. Suddenly it all makes sense. Throw a piece of impure gold into melted stibnite, Principe explains, and it dissolves very rapidly, producing a brilliant white alloy.

Again and again, Principe melds rich historical erudition with deft chemical manipulation. The results are always convincing and sometimes—as when one recipe yields a golden object in the form of a tree—breathtaking. And the upshot of it all is clear. Newton and his fellow aficionados, who included Boyle, Hooke, and Leibniz, had every reason to believe that alchemy might yield profound knowledge about nature. They had not only read enticing texts and heard astonishing stories by the dozen but also witnessed dramatic demonstrations of what chymistry—Principe’s and Newman’s term for what they practiced—could actually do.

In the end—and here Principe’s historical work is at its most subtle and acute—the point is not sim-



An Alchemist in His Workshop (David Teniers II, 17th century).

ply that alchemy was empirical. For it wasn't a purely experimental pursuit. The alchemists took their symbols and systems as seriously as the metallic crystals they fabricated. They saw the universe as a complex set of networks, of substances and objects tied together by "secret knots" and occult forces. Only those who combined the skills of the artisan with the insight of the inspired philosopher could make new substances. And Newton and his contemporaries inherited this approach. To that extent, Keynes was right to describe Newton as "Copernicus and Faust all in one."

References

1. J. M. Keynes, in the Royal Society, *Newton Tercentenary Celebrations: 15–19 July 1946* (Cambridge Univ. Press, Cambridge, 1947), pp. 27–34.

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EXHIBITION

Remember— You Will Die

Caroline Ash

The Wellcome Collection's current exhibition *Death* originates in objects amassed by the entrepreneurial print collector Richard Harris, who over the years has gathered and sold several notable assemblages of rare objects. This one started as a collection of skulls and skeletons, which, Harris admits, has become a contemplation of his own demise. Even the limited selection of objects on display was so obviously acquired to fulfill his particular taste that the exhibition could be viewed as a practice mausoleum. Aptly enough, the first piece Harris procured for the collection is a *vanitas* by Adriaen van Utrecht (1599–1652)—a still life depicting the temporary nature of life symbolized by flowers, coins, and timepieces with the triumphant permanence of death shown as a laurel-crowned skull. The human skull, a universal symbol for death, forms the exhibition's dominant motif. The many wonderful, shocking, and humorous images on display are organized around five themes: contemplation, dance, violence, eros and thanatos, and commemoration.

We are first invited to contemplate death by a series of memento mori, which range from printed bookplates to Albrecht Dürer's beautiful portrait of St. Jerome. Death was not always the enemy; sometimes it's a friend, and sometimes it's playful. The skeleton musicians in the woodcuts of Guyot Marchant's



Mondongo's *Calavera*.

edition of *Danse Macabre* (1485) remind us how life is played to Death's tune. Interestingly, older pieces show revived corpses as near skeletons still respectfully clad in skin. Quite often Death can be ludicrous. Two panels of robustly frolicking skeletons by Kawanabe Kyōsai (1831–1889) illustrate life's absurdity and the problem of collapsing parts. Late-19th-century photographs of dissections were always strange and sometimes blackly humorous, and contemporary artist John Isaacs's *Are You Still Mad at Me* (2001) is hysterically funny and too gruesome to contemplate. Not an anatomical piece, this sculpture is more a hatchet job, and once you realize what it's about, any sense of mirth becomes panic.

Not always solely about death, depictions of skulls can symbolize a variety of emotional and political states of the living. A huge plasticine sculpture by the Argentinian collective Mondongo, *Calavera* (2011), depicts the anger of the dispossessed at the dominance of northern economic and cultural power. Likewise, a print by the German artist Käthe Kollwitz (1867–1945) displays her fury at social injustice by illustrating it as rape by Death. In an 1896 etching, the Belgian symbolist Félicien Rops also chose to use a sexual act with Death as a way of interpreting St. Theresa's religious ecstasy.

There is a lot of great art on display. George Grosz's 1958 collage of skulls on living persons declares the anonymizing effect of death, also a strong theme of Otto Dix's and Francisco Goya's series of alternately shocking and moving anti-war prints. There's a portrait of Robert Mapplethorpe's skull-headed cane that was memorialized by the photogra-

pher a year before his death from AIDS. But there's not much science, except obliquely.

So unfortunately, the eclecticism in this venue is ultimately disappointing. Here was a fantastic opportunity to integrate Harris's fine art pieces with objects from the Wellcome Collection in imaginative ways. Within the exhibition, science is most notably symbolized by the model of the skull of the living artist Kiki Smith. Coordinates of her skull obtained through medical-imaging technology were used to cast a small, delicate bronze cranium—a poignant symbol of the neoteny of modern humans. The combination of the concept and the process used to create this piece would make it a candidate for a more typical Wellcome exhibition. The Visible Human teaching aid and *White's Physiological Manikin* (1899) supply some anatomy. The latter is an impressive object: a life-size book composed of chromolithographed sheets that fold out to reveal various components of the human body. Intriguingly, it shows limbs in

muscular cross section like the branches of a tree, and it recapitulates Hans Sebald's 1543 depiction of the tree of life as a skeleton. One concession to plain science is a "blobbogram" at the end of the exhibition that gives us a view of what kills humans most:

infectious diseases now just being eclipsed by long-term chronic disease and closely followed by human beings themselves.

Death is more than skulls. What I had expected to learn from this exhibition was how scientists and artists have united to visualize death phenomena, such as programmed cell death and necrosis or decomposition and microbial resurrection. What about the psychology and anthropology of grief, mourning, and graveyards; the science of embalming and mummification; the pathologist's and forensic scientist's jobs; the issues surrounding assisted death; and so on? There are many ways to meet death. However, there are several accompanying events (*I*)—including films, interviews, talks, and performances—that culminate in "What makes a good death?" (an evening of literary readings followed by a day of talks and discussion, 1–2 February. Together, these should give a more satisfying account of this universally absorbing subject than the exhibition alone.

References

1. www.wellcomecollection.org/whats-on/exhibitions/death-a-self-portrait/events.aspx.

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Interdisciplinary Graduate Training in Teaching Labs

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Intensive, short-term courses meld students and faculty and new techniques in pursuit of genuine research questions.

Modern research and training in the life sciences require cross-disciplinary programs, integrating concepts and methods from biology, physics, chemistry, and mathematics. We describe the structure and outcomes from an example of one such approach, the Physiology Course at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, and discuss how similar intensive, team-building research courses are also being applied to improve graduate education in universities. These courses are based on teaching laboratories that have students address contemporary research questions by combining ideas and approaches from biology, computation, and physics.

Bringing biologists and physical and computational scientists together does not automatically translate into collaborations and novel insights into biological problems. Perhaps the best solution is to merge physical and life sciences training during undergraduate studies (1–4). Yet there will always be a need to teach physical sciences to biologists, and vice versa, during graduate and postdoctoral training, as interests and motivations change over time. Learning scientific inquiry in a teaching laboratory has been implemented successfully for undergraduates (5–8), but is not common practice in graduate education, where lecture courses tend to occupy the first year followed by the pursuit of an individually directed thesis project.

Boot Camp and Real Problems

The summer Physiology Course at the MBL, founded in the 1890s by Jacques Loeb, has a rich history of training scientific leaders and gathering teaching faculty from diverse disciplines in ways that did not typically occur

in university departments. Although focused on basic biological research, the Physiology Course has long admitted physicists, engineers, mathematicians, and chemists. In 2004, the interdisciplinary nature of the course became less adventitious and more deliberate when the course directors decided to draw an approximately equal number of the ~28 U.S. and international students from cell biology and physical science/computational backgrounds. Special interdisciplinary grad-

uate training programs have been established to bridge gaps between disciplines [e.g., those funded by the Integrative Graduate Education and Research Traineeship (IGERT) from the U.S. National Science Foundation (NSF)], but the goal of the Physiology Course is to provide a short-term (7-week), intensive (6 days/week; ~14 hours/day) environment for interdisciplinary education.

... course culture must minimize fear of failure ...

Three ingredients have been particularly important for the Physiology Course: (i) promoting exploration of new techniques at the start of the course; (ii) focusing the laboratory on real research questions; and (iii) promoting collaboration and peer-to-peer learning, which creates benefits that extend beyond the course by fostering longer-term scientific social networks. The third component has been discussed elsewhere (9), so we focus on the first two elements below.

Boot camp: Leveling the playing field for learning. To expose students to new subjects and techniques, the Physiology Course begins with a 6-day “boot camp,” in which students rotate through intensive 2-day modules in “wet bench” biochemistry, light microscopy and image analysis, and MATLAB programming. Computational scientists find themselves dissecting squid optic lobes and purifying proteins. Biology students discover that they can write simple code for identifying subcellular organelles in images or using differential equations to analyze population dynamics. Although 2 days is too short to become expert in such skills, the boot camp allows students to

outcomes, students and faculty combine their wits and skills to design experiments, evaluate progress, and troubleshoot along the way. Curiosity, the excitement of possible discovery, and the challenge of problem-solving can be seductive for students. Real problems teach students that science is difficult and that nature does not yield its secrets easily. Such lessons are valuable and not necessarily discouraging.

Every Physiology Course student participates in three research modules, led by a faculty member and postdoctoral or graduate student teaching assistants, each consisting of 11 days for experimentation and analysis and a final day for presentations. The faculty members bring project ideas (usually nascent ideas never tested in their labs), and a given project is typically tackled by two to four students with mixed backgrounds in biology and physical science and computation. The course projects, which differ every year, are tailored to this time period, and many of the reagents (e.g., clones or cell lines) are prepared in advance. Students are encouraged to divert these initial projects in new directions or come up with their own ideas and approaches. The result is a “hybrid vigor” of ideas, which often improves upon the original experimental vision brought by the faculty.

Advantages and Outcomes

How might a graduate student benefit from a teaching laboratory when the majority of her or his training is already spent doing

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thesis research? A teaching lab of the kind described here emphasizes strategic decisions that are important for completing a thesis and becoming a professional scientist: how to formulate good research questions, develop strategies to answer those questions, and overcome the inevitable obstacles. However, the focus and intensity of the teaching lab differ from typical thesis work; students brainstorm and troubleshoot with faculty and peers on a daily basis in the lab instead of during sporadic thesis meetings in an office and discover that they can make considerable progress in a brief time span. Although intensive, a good teaching lab provides a playful setting for students to learn and experiment with new ideas and techniques, which can be difficult to do in their focused thesis work. Students also experience the intimacy of interacting with peers and faculty members in an unusual collaborative setting, which can generate a passion for the scientific profession that persists after the course ends.

A teaching laboratory also is effective for interdisciplinary training, as physics and biology students can become more motivated to grapple with a new method or principle when they see its relevance to a problem they are trying to solve. Physics and computational students experience firsthand the complexity and “messiness” of experimental biology. They learn how to identify a good biological question and set up controlled experiments to answer it, a process that differs somewhat from the “observations plus modeling” approaches at the core of many physical science disciplines. Biology students gain exposure to applying quantitative thinking and embracing a broader range of tools (e.g., microfluidics, mathematics, computer simulations, chemical kinetics, and optical trapping). By working intensively together, biologists and physicists better understand each other’s languages and how their approaches can complement one another.

To be successful in achieving such training goals, the laboratory course culture must minimize the fear of failure or of appearing ignorant, factors that impede students, as well as senior scientists, from venturing into new fields or learning new approaches. Risk-taking must be part of the course philosophy. Leading by example, the faculty stretch themselves to learn new material, as students watch them, too, fumble with a computer program or a microscope. Traditional roles that separate teacher and student become blurred, as the two groups work on a challenging research problem rather than being

separated at opposite ends of a lecture hall.

Physiology Course students are encouraged to take risks and try new methods without aiming for publishable results. However, by focusing on interesting questions as the core philosophy of the Physiology Course, important ideas and fruitful research have emerged, as evidenced by the 23 research papers and 59 meeting abstracts that cite the Physiology Course, as part of the author affiliation or acknowledgments between 2005 and 2012 [supplementary materials (SM)]. In addition, 78 out of 176 students polled (44%) reported that they continued to work on some experiment or question to which they were initially exposed during the course (SM). Our surveys also show that students favorably view the research-based teaching lab (SM), but rigorous evaluation of longer-term career benefit remains an important future goal.

Influences, Translation, and Challenges

The MBL and Cold Spring Harbor Laboratory (CSHL) have become influential educational centers because they have dedicated and well-equipped laboratory space for teaching, on-site housing and meals for students and faculty, and public and private funding to enable advanced research. However, laboratory courses with the same flavor can be created for graduate education in a university setting.

The integrative Program in Quantitative Biology (iPQB) at the University of California, San Francisco, inspired by the project-based instruction at MBL and CSHL courses, provides an interdisciplinary laboratory experience for incoming Ph.D. students from physical science, engineering, computer science, and biology backgrounds. The first-year curriculum begins in a teaching lab with a week-long “boot camp,” followed by a team-based intensive experimental and computational course in which they pursue faculty-guided, genuine research problems. The curriculum includes “Team Challenges,” such as the construction of a “breadboard” fluorescent or hyperspectral microscope from basic glass lenses and parts, which they then use for single-cell analyses. In the second quarter, teams computationally model and explore the experimental data that they generated in the first quarter. With similar inspiration, the California Institute of Technology (Caltech) launched a 1-week Physical Biology boot camp with a research component. In addition to Caltech undergrad and graduate students, the course has attracted non-Caltech postdocs, professors, journal editors, and policy-makers.

Similar educational efforts can be tried in universities if scientist-educators are freed from traditional constraints of trimester or semester academic calendars where several courses run in parallel to make room for courses that operate on compact, intensive schedules (10). The spirit of the course (collaborative work between students and faculty to address real research questions, emphasizing experimentation, modeling, and active discussion) can be created without the resources available at the institutions described here. The degree of challenge (complexity of the questions being tackled) can be tuned to accommodate the resources and time available. If a dedicated teaching lab is not available, faculty might open up their labs to students as we (R.D.V. and R.D.M.) have done for short (2-week) courses at UCSF.

A challenge for graduate education is to develop courses that excite both students and faculty and that pave the way for independent thesis work and subsequent careers in research. Graduate courses that break from the undergraduate lecture model should aim for three goals: (i) to signal the transition from absorbing knowledge (undergraduate) to creating knowledge (graduate school); (ii) to do a better job of teaching the skills needed to become a practicing scientist; and (iii) to energize and engage faculty members. The teaching labs we describe here represent one of many creative approaches that can be used to tailor education toward the unique needs of science graduate students.

References and Notes

1. W. Bialek, D. Botstein, *Science* **303**, 788 (2004).
2. J. B. Labov, A. H. Reid, K. R. Yamamoto, *CBE Life Sci. Educ.* **9**, 10 (2010).
3. H. J. Chiel, J. M. McManus, K. M. Shaw, *CBE Life Sci. Educ.* **9**, 248 (2010).
4. P. Pevzner, R. Shamir, *Science* **325**, 541 (2009).
5. D. I. Hanauer *et al.*, *Science* **314**, 1880 (2006).
6. J. Chen *et al.*, *PLoS Biol.* **3**, e59 (2005).
7. National Research Council, *Biol.2010: Transforming Undergraduate Education for Future Research Biologists* (National Academy of Sciences Press, Washington, DC, 2003).
8. A. E. Bednarski, S. C. Elgin, H. B. Pakrasi, *Cell Biol. Educ.* **4**, 207 (2005).
9. M. K. Smith *et al.*, *Science* **323**, 122 (2009).
10. A. M. Bentley, S. Artavanis-Tsakonas, J. S. Stanford, *CBE Life Sci. Educ.* **7**, 175 (2008).

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Supplementary Materials

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BIOCHEMISTRY

Visualizing the Influenza Genome

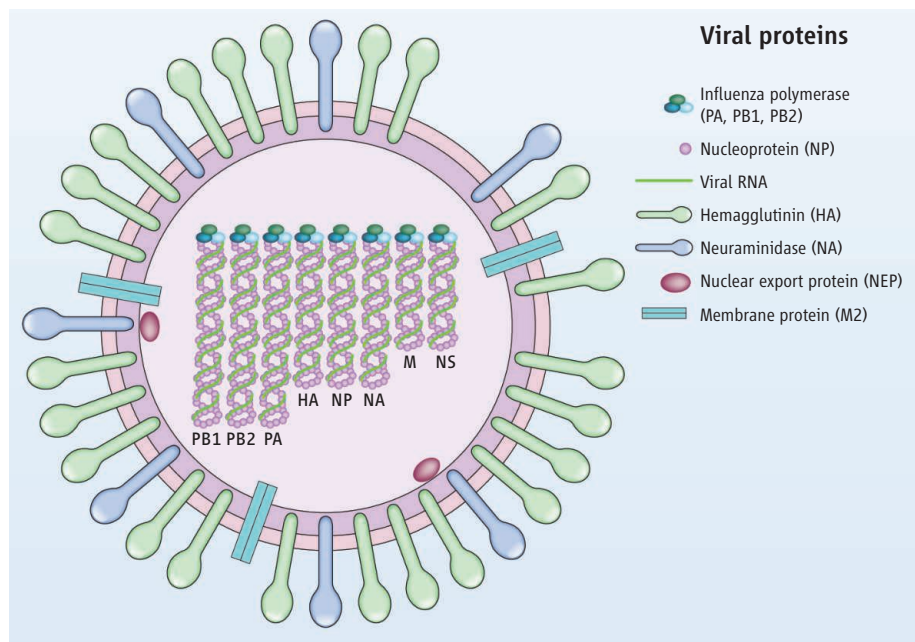
Yitzhi Jane Tao and Wenjie Zheng

Cellular organisms have evolved complex mechanisms to maintain and regulate genome structure and dynamics. Viruses are no exception. On pages 1634 and 1631 of this issue, Arranz *et al.* (1) and Moeller *et al.* (2) report three-dimensional views of the genomic RNA-protein complex of the influenza A virus, the infamous pathogen responsible for human flu epidemics and pandemics. These findings should help to elevate our understanding of influenza virus structure and biology to a new level.

Through a technique called three-dimensional electron microscopy (EM) reconstruction, the two research groups obtained stunning images of a double-helical hairpin structure, formed by the genomic RNA of the influenza A virus in complex with several viral proteins. The genome of influenza A viruses consists of eight segments of negative-sense, single-stranded RNA organized as individual ribonucleoprotein (RNP) complexes (see the figure). Each RNP contains a stoichiometric amount of the viral nucleoprotein (NP), a single copy of the viral polymerase, and a viral RNA. At ~ 20 Å resolution, the results show clearly that the NPs follow a double-helical path in RNP, forming an internal protein scaffold with five or six pairs of NP molecules in each turn.

The authors generated pseudo-atomic models for the double-helical NP scaffold by fitting a NP crystal structure (3) into the density maps. Assuming that the positively charged groove of NP serves as the RNA binding site, Arranz *et al.* and Moeller *et al.* generated complete models for the double-helical region of the viral RNP. They show that around 120 to 150 RNA nucleotides are accommodated in each helical turn. Earlier EM studies of the influenza virus RNPs suggested double-helical structures, but the new findings elucidate the structural basis of such double-helical structures at the molecular level.

To understand the structural arrangement of the RNP of influenza A, it is useful to draw an analogy with the double-helical DNA duplex. Similar to the DNA duplex, RNP exhibits a major and a minor groove on its surface. However, the cohesion of



Influenza A virus. The virus, with a diameter of ~ 100 nm, contains an outer membrane envelope and an inner matrix protein layer that enclose a single-stranded, segmented RNA genome. Each viral RNA segment is folded into a double-helical hairpin structure, as shown by the three-dimensional images obtained by Arranz *et al.* (1) and Moeller *et al.* (2). These double-helical hairpin structures, called the ribonucleoprotein complexes, play critical roles in virus assembly and infection.

the structure is maintained differently: In a DNA duplex, cohesion between the two antiparallel strands is maintained by base pairing, whereas the interaction between the two opposing arms of an RNP hairpin is solely mediated by the NP molecules. Interactions between adjacent NP molecules on the same RNA strand are facilitated by the extended tail loop of NP. The double-helical RNP is ~ 15 nm wide and estimated to be ~ 65 nm long for gene segment M, which encodes two viral proteins (M1 and M2) and contains ~ 1000 nucleotides (1) (see the figure).

The detailed RNP structures reported by Arranz *et al.* and Moeller *et al.* will have a tremendous impact on our understanding of influenza virus transcription, replication, RNP intracellular transport, and virus assembly. Because the RNP structure is mostly maintained by the NP protein and the bound RNA is fully solvent-exposed, the viral polymerase moving along the RNA template during viral RNA synthesis should result in little or only local disruption in the double-helical hairpin structure. Indeed, it was previously noted that the dissociation of

bound RNA had little effect on the overall structure of the RNP (4).

The new structures show that the viral polymerase occupies the open end of the RNP hairpin, where it simultaneously interacts with both the 5' and 3'-ends of the viral RNA. At the other end of the RNP hairpin is a small loop formed by a curved array of three to eight NP molecules. The influenza virus polymerase is a heterotrimeric complex with multiple enzymatic and ligand-binding activities. Crystal structures have been reported for several fragments of the viral polymerase (5), but the relative orientation of these domains in the complex is not well understood. The RNP structures obtained by Arranz *et al.* and Moeller *et al.* offer a unique opportunity to determine how these polymerase domains interact with each other, with NP, and with RNA to mediate transcription and replication of the viral genome.

The work by Arranz *et al.* and Moeller *et al.* represents a major technical advance in structural studies of short helical assemblies. Three-dimensional reconstruction of the influenza virus RNP has long been considered

challenging because of its small size, short length, high flexibility, and structural instability; many investigators questioned whether RNP has a well-defined helical symmetry.

The two research groups accomplished this seemingly impossible task with the iterative helical real-space reconstruction method (6), in which the central helical region was divided into small fragments and initial helical parameters were determined from non-averaged maps. The two termini of the RNP were separately extracted and reconstructed. As a result, the RNP structures reported in both papers are montages assembled from three independent reconstructions. Variations are noted in the helical parameters and the NP orientations of the two RNP models reported by the two groups. Possible explanations for

intermodel variations include the model resolution, different handedness of the electron density maps, rotational freedom of the NP molecules, and source of RNP samples (viral particles versus cells).

The work by Arranz *et al.* and Moeller *et al.* greatly expands our horizon by establishing reliable methods for the isolation and structural characterization of the influenza virus RNPs. Further studies of this kind would allow scientists to address many long-standing questions in the flu field. For instance, how does the helical structure of RNP rearrange during viral RNA transcription and replication? When do newly synthesized RNPs adopt the double-helical morphology? And how do the eight RNPs of influenza A interact with each other to mediate genome packaging

and gene reassortment? Novel biochemical approaches combined with ever-improving computational and structural techniques should help to uncover much-needed insights into these complex yet intriguing problems.

References and Notes

1. R. Arranz *et al.*, *Science* **338**, 1634 (2012); 10.1126/science.1228172.
2. A. Moeller *et al.*, *Science* **338**, 1631 (2012); 10.1126/science.1227270.
3. Q. Ye, R. M. Krug, Y. J. Tao, *Nature* **444**, 1078 (2006).
4. R. W. Ruigrok, F. Baudin, *J. Gen. Virol.* **76**, 1009 (1995).
5. P. Resa-Infante *et al.*, *RNA Biol.* **8**, 207 (2011).
6. E. H. Egelman, *Curr. Opin. Struct. Biol.* **17**, 556 (2007).

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HISTORY OF SCIENCE

Tackling Meningitis in Africa

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The United States is currently in the midst of a meningitis outbreak (with at least 36 deaths) as the result of fungal contamination of steroid injections to relieve back pain (1). In Africa's so-called "meningitis belt," outbreaks of meningitis are a regular occurrence, killing thousands and infecting tens of thousands each year. In 2009, about 5300 people died of meningitis and 88,000 were infected with the disease (2). The meningitis belt stretches from Senegal in the West to Ethiopia in the East and includes around 300 million people. Sanofi Pasteur had provided Africa with a meningitis vaccine for decades but because of reduced supplies in 2006 and 2007, and a threat of increased incidences of the disease, the World Health Organization (WHO) made a call for additional vaccine providers (3). But it wasn't multinational companies from wealthy nations that responded, but two Latin American countries that answered the call. What brought Brazil and Cuba together in this seemingly unlikely collaboration?

It was the entrepreneurial arms of two public research organizations in Brazil and Cuba—Bio-Manguinhos and the Finlay Institute, respectively—that jointly proposed to manufacture a polysaccharide vaccine against meningitis (serotypes A and C).

In recognizing that this partnership could indeed meet the demand, the WHO supported the alliance and prequalified their vaccine. The venture has since supplied more than 19 million doses (4), distributed through the WHO, United Nations Fund for Children (UNICEF), Doctors Without Borders, and the International Red Cross, among other organizations.

This apparently straightforward account of how two enterprises came together to provide a much needed treatment is based on a relationship that developed over time. The ability of both countries to produce vaccines had been built over decades (5–8). Cuba, with a relatively strong record of innovation, had created a synthetic vaccine against *Haemophilus influenzae* type b (Hib) and a meningitis BC

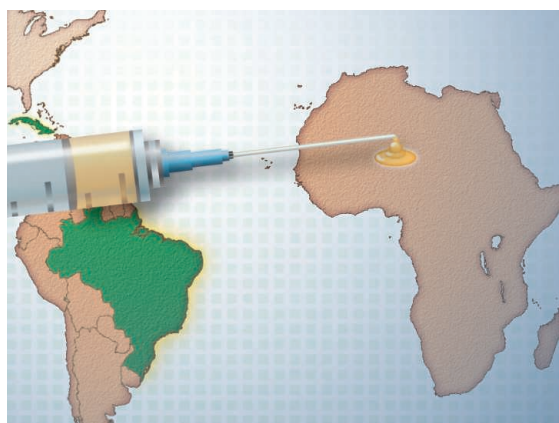
How did two Latin American countries harness their scientific strengths to address a major medical problem in Africa?



vaccine (9); its expertise was key to providing the active ingredients of the meningitis AC vaccine. Vaccine production was the strength of Bio Manguinhos; its use of lyophilization (freeze-drying) improved vaccine stability, storage, and transport. By harnessing their respective capabilities, Bio-Manguinhos and the Finlay Institute could together respond fast and effectively to the meningitis outbreak. Although neither country had suffered serotype A meningitis, they could respond to an outbreak elsewhere.

The production capacities of Bio-Manguinhos and the Finlay Institute also allowed them to provide a relatively inexpensive meningitis vaccine. Their price was US\$ 0.95 a dose compared to \$15 to \$20 a dose for polysaccharide vaccines against serogroups AC, W135, and Y on the international market, and \$80 a dose for a conjugated meningitis vaccine used in high-income countries (4, 10) (the latter type provides broader protection and a longer immunization period). Because the Brazil-Cuba vaccine targeted the meningitis strain in Africa, the two organizations had to develop an inexpensive health product accessible to the local populations.

Another reason for the success of the Brazil-Cuba venture



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was their prior work together in the biopharmaceutical sector. Since the late 1990s, the governments of Brazil and Cuba had been steadily promoting scientific interaction to emphasize South-South collaboration. This sector was singled out for partnerships given each nation's strengths in this arena as well as some common health problems such as tropical diseases and a growing noncommunicable disease burden (including cardiovascular disease and cancer). Together, they had an applied focus—to increase the availability of affordable health products that serve local health needs. Bio-Manguinhos had already collaborated with several Cuban biotechnology institutes and, for example, transferred affordable technologies from Cuba to produce interferon alpha-2b and erythropoietin. The main benefits of these joint projects were savings for Brazil's public health system and income from royalties for Cuba. These earlier, mutually beneficial collaborations were a strong foundation to build upon.

The Brazil-Cuba meningitis project was not their only collaboration to have benefited a third party. They are now jointly promoting health and development in Haiti following the 2010 earthquake and will construct hospitals, support immunization programs, and strengthen laboratories for disease surveillance in Haiti (11).

Most international collaborations include elements of self-interest and desires for the alliance to benefit all parties concerned. Sometimes developing countries are transparent about this and “tie” their aid by demanding that products and services be supplied exclusively by them instead of being purchased on the international market. China and India, for example, tie their aid to Africa by requesting that services and products in their African initiatives be supplied by them (12, 13). However, South-South and North-South approaches to assistance can be viewed as having different philosophies. Whereas the latter is generally grounded in altruism, the former is built on solidarity between countries that have had to survive under challenging conditions. This distinction sets a different tone for South-South collaboration. The solidarity among developing countries began to surface in the 1950s during the quest for independence from colonial powers, and many developing nations sought alternatives to dealing with the North to address issues of concern. Today, there is an increasing scope for South-South interaction, as the developing world becomes technologically proficient and experiences economic growth (14). South-South partner-

ships are therefore promising for tackling many shared challenges in health, agriculture, and environmental protection. Given that aid from traditional Northern donors is declining with the continuing global economic recession (15), international and philanthropic organizations, and governments in high-income countries, should recognize South-South enterprises to a larger extent in strategies that promote global health and development.

References and Notes

1. CDC, “Multistate Fungal Meningitis Outbreak - Current Case” (Centers for Disease Control and Prevention, Atlanta, GA, 2012).
2. WHO, in *Fact Sheet Number 141* (World Health Organization, Geneva, 2011).
3. IRIN, “Africa: Fighting meningitis a race against time” (2007); www.irinnews.org/fr/Report/70740/AFRICA-Fighting-meningitis-a-race-against-time.
4. T. W. Sáenz, M. C. Souza-Paula, M. Ray, H. Thorsteinsdóttir, in *South-South Collaboration in Health Biotechnology: Growing Partnerships Amongst Developing Countries*, H. Thorsteinsdóttir, Ed. (International Development Research Centre and Academic Foundation, Ottawa, 2012).
5. H. Thorsteinsdóttir, T. W. Sáenz, U. Quach, A. S. Daar, P. A. Singer, *Nat. Biotechnol.* **22** (suppl.), DC19 (2004).
6. S. M. Reid-Henry, *The Cuban Cure* (Univ. of Chicago Press, Chicago, 2010).
7. M. Ferrer, H. Thorsteinsdóttir, U. Quach, P. A. Singer, A. S. Daar, *Nat. Biotechnol.* **22** (suppl.), DC8 (2004).
8. R. Rezaie *et al.*, *Nat. Biotechnol.* **26**, 627 (2008).
9. F. Sotolongo Padron *et al.*, *MEDICC Rev.* **9**, 18 (2007).
10. P. Grogg, “Cuba, Brazil unite for Africa's health,” South-South Solutions, Media Briefs, 2010, UNDP and Inter Press Service News Agency.
11. Cooperação Internacional Tripartite Brasil, Cuba, Haiti (2012); <http://cooperacaotripartitehaiti.tumblr.com>.
12. D. Brautigam, *The Dragon's Gift* (Oxford Univ. Press, Oxford, 2009).
13. A. K. Kapoor, P. Singer, J. Wong, H. Thorsteinsdóttir, in *South-South Collaboration in Health Biotechnology: Growing Partnerships Amongst Developing Countries*, H. Thorsteinsdóttir, Ed. (International Development Research Centre and Academic Foundation, Ottawa and New Delhi, 2012), pp. 233–268.
14. UNDESA, “Development cooperation for the MDGs: Maximizing results” (United Nations Department of Economic and Social Affairs, New York, 2010).
15. P. Love, “Development aid drops for the first time in 15 years.” *OECD Insights* (2012); <http://oecdinsights.org/2012/04/04/development-aid-drops-for-the-first-time-in-15-years/>.

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EVOLUTION

Splicing in 4D

Panagiotis Papasaikas^{1,2} and Juan Valcárcel^{1,2,3}

Flexibility in regulating RNA splicing can generate diverse phenotypic differences among equivalent organs across vertebrates.

In the chapter of *The Origin of Species* entitled “Difficulties on Theory,” Charles Darwin found it “most difficult to conjecture by what transitions an organ could have arrived at its present state.” On pages 1587 and 1593 of this issue, Barbosa-Morais *et al.* (1) and Merkin *et al.* (2) advance our understanding of the molecular mechanism by which the genome generates differences in organs between species. This part of the answer relies on the broken syntax of genomic messages and uncovers striking differences in how evolution shapes the different layers of gene regulation.

Genes in eukaryotic organisms are first transcribed as precursor messenger RNAs (pre-mRNAs) in which “meaningful” sequences (exons), which code for amino acids or harbor regulatory sequences, are interrupted by (usually) longer pieces

(introns) that are removed by splicing. The resulting mature mRNAs are then translated into proteins, which carry out enzymatic and structural functions in the cell. Remarkably, different cell types can interpret the same sequence of a pre-mRNA either as an exon or as an intron. This leads to different patterns of splicing that represent cell type-specific alternative interpretations of the genomic information. Alternative splicing allows the shuffling of protein-coding domains or confers distinct sensitivity of the spliced mRNAs to regulatory factors (3). Thus, gene transcription and alternative splicing provide separate mechanisms by which particular cell types can determine the complement of proteins required for carrying out their specialized functions in the organism.

Patterns of tissue-specific gene activation are highly conserved among vertebrates. Indeed, Merkin *et al.* find that only when considering long evolutionary periods (e.g., 300 million years after the split between birds and mammals) can a species-specific signa-

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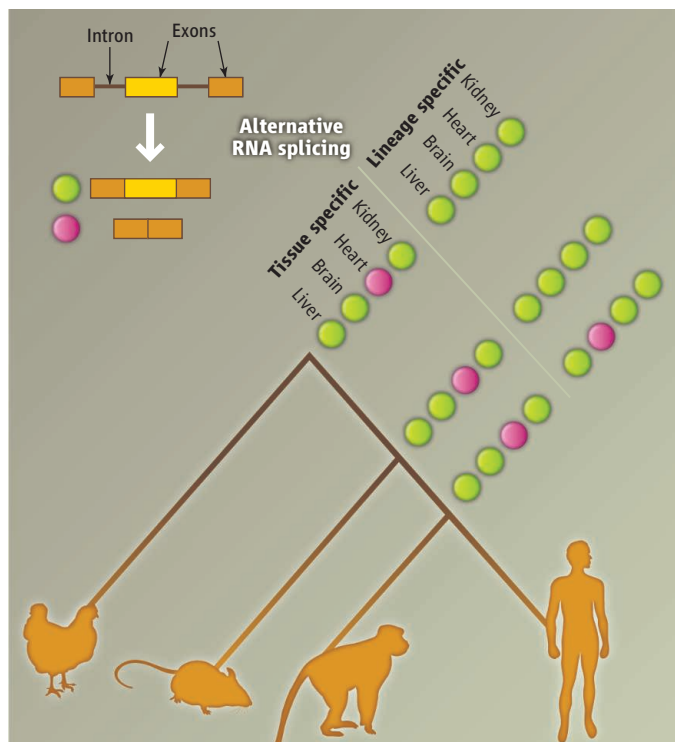
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ture of gene transcription be seen to dominate over the highly conserved tissue-specific signatures. What could then be the source of genome-originating divergence leading to differences in organs between species?

Previous comparisons indicated that conservation of alternative splicing patterns in orthologous genes is limited (4). For example, only 10% of exons conserved between mouse and human display alternative splicing in both species (5). The general lack of phylogenetic conservation has been considered an argument against the functional relevance of most alternative splicing events. The results of Barbosa-Morais *et al.* and Merkin *et al.*, however, bring a fresh perspective to this issue because both studies reveal that alternative splicing and transcription regulation are under very different evolutionary constraints. Indeed, these studies find that alternative splicing patterns are dominated by species-specific differences that accumulate even during relatively short evolutionary periods of 6 million years, implying that tissue-specific splicing diverges in particular lineages at a pace one to two orders of magnitude faster than transcriptional changes (see the figure).

Nevertheless, Barbosa-Morais *et al.* and Merkin *et al.* uncovered a group of a few hundred alternatively spliced exons whose tissue-specific regulation is highly conserved, in some cases over periods of hundreds of millions of years. The unprecedented resolution of the high-throughput RNA sequencing data provides a wealth of information that, together with results from other technologies (6), will add to our appreciation of the diversity and potential functions of splicing regulation in organogenesis.

The complex molecular machinery of the spliceosome recognizes the sequence boundaries between exons and introns and mediates intron removal (7). Regulation of splice site choice involves the interplay between numerous regulatory sequence motifs present in introns and exons and the trans-acting factors that recognize these sequences to promote or prevent spliceosome assembly on particular splice sites (8, 9). Merkin *et al.* discovered enrichment in binding sites for well-known regulators of alternative splicing during cell differentiation, uncovering an ancestral splicing code.



Shortly after the discovery of introns, the molecular biologist Walter Gilbert speculated that the “mosaic” intron-exon architecture of genes could accelerate evolution (10). Gilbert reasoned that incremental mutational steps near the intron-exon boundaries could lead to the coexistence of alternative transcripts and therefore allow exploration of sequence space without radically disrupting the previous gene function. The results of Barbosa-Morais *et al.* and Merkin *et al.* support this concept as a general evolutionary strategy adopted by vertebrates, reporting frequent conversion between alternative and constitutive splicing and also an increase in the prevalence of alternative splicing in primates. This plasticity can be attributed to evolutionary tinkering with regulatory sequence motifs, as demonstrated by Barbosa-Morais *et al.* using mouse cells engineered to harbor human chromosome 21. In these cells, human transcripts maintain their distinct patterns of alternative splicing despite being produced by the mouse splicing machinery. The regulatory flexibility and constant flux of alternative splicing exploits the combinatorial interplay and positional effects of a relatively small number of generally ubiquitous splicing regulators (8, 9). This is again in contrast to tissue-specific transcription, which relies on a larger set of tissue-specific activators (8).

Barbosa-Morais *et al.* and Merkin *et al.* showcase ways in which vertebrates can capitalize on the potential of split gene organi-

Splicing and species diversification.

Although conserved tissue-specific patterns of alternative exon usage exist, most differences in alternative splicing patterns are distinct between evolutionary lineages and can contribute to phenotypic divergence of organs in different vertebrate species.

zation for adding, removing, or altering specific aspects of transcript functionalities. Merkin *et al.* provide a compelling example in the control of protein phosphorylation: Exons that are differentially included among tissues encode protein segments enriched in phosphorylation sites. Differential inclusion of these exons is more prominent in those tissues that express the enzymes (kinases) that phosphorylate these sites, suggesting that differential splicing—rather than kinase amounts—predominantly modulates the extent of protein modification. Enrichment

in phosphorylation sites is also observed in alternative exons with highly variable tissue distribution among species, implying a role for alternative splicing in the modification of kinase signaling circuits between species.

Barbosa-Morais *et al.* additionally indicate that species-specific splicing is frequent in regulatory gene encoding nucleic acid binding proteins, and that both tissue- and species-specific exons typically correspond to unstructured regions of proteins that are often involved in protein-protein interactions (11, 12). Together, the two studies underscore the multidimensional impact of alternative splicing by modulating the scope of signaling, gene regulation, and protein-protein networks both in tissue differentiation and during evolution.

References

1. N. L. Barbosa-Morais *et al.*, *Science* **338**, 1587 (2012).
2. J. Merkin, C. Russell, P. Chen, C. B. Burge, *Science* **338**, 1593 (2012).
3. A. Kalsotra, T. A. Cooper, *Nat. Rev. Genet.* **12**, 715 (2011).
4. H. Keren, G. Lev-Maor, G. Ast, *Nat. Rev. Genet.* **11**, 345 (2010).
5. Q. Pan *et al.*, *Trends Genet.* **21**, 73 (2005).
6. J. C. Castle *et al.*, *Nat. Genet.* **40**, 1416 (2008).
7. M. C. Wahl, C. L. Will, R. Lührmann, *Cell* **136**, 701 (2009).
8. M. Chen, J. L. Manley, *Nat. Rev. Mol. Cell Biol.* **10**, 741 (2009).
9. Y. Barash *et al.*, *Nature* **465**, 53 (2010).
10. W. Gilbert, *Nature* **271**, 501 (1978).
11. M. Buljan *et al.*, *Mol. Cell* **46**, 871 (2012).
12. J. D. Ellis *et al.*, *Mol. Cell* **46**, 884 (2012).

MEDICINE

Cardiac Regeneration

Anthony Rosenzweig

In the United States, heart failure afflicts about 6 million people (1), costs \$34.4 billion each year (2), and is now the single most common discharge diagnosis in those over 65 (3). Although enormous progress has been made in managing acute cardiovascular illnesses such as heart attacks, many patients go on to develop late sequelae of their disease, including heart failure and arrhythmia. Thus, the growing number of these patients in some ways represents a burden of our success. It also reflects the incomplete success of most current therapies, which mitigate and manage but do not cure the disease.

Decades of clinical research support interventions that improve outcomes in heart failure patients, including beta-blockers, angiotensin-converting enzyme inhibitors, and mineralocorticoid antagonists. These medicines block pathways that are likely compensatory initially but become progressively more maladaptive. Running the engine in hyperdrive appears to contribute to both dysfunction and loss of cardiomyocytes—the dynamically contractile cells essential for heart function—with dire clinical consequences. Disrupting this cycle has proven clinical benefits but mostly seems to slow disease progression. Consequently, prognosis and quality of life remain poor for many heart failure patients (4, 5).

Traditionally the adult heart was viewed as having virtually no capacity for cardiomyogenesis to compensate for losses occurring in heart failure (6, 7) and other cardiac diseases. However, animal and human studies now support a more dynamic model in which the heart has some endogenous regenerative potential (8–12). New cardiomyocytes may arise from existing cardiomyocytes (9, 13, 14) and progenitor or stem cells (8, 10). Although the relative contribution of these lineages is debated and likely varies in different settings, the recognition of even limited regenerative capacity in the heart is exciting both for what it tells us about the body's plasticity and for its therapeutic potential.

Multiple cell therapy trials have been published, and several themes emerge. One is that while issues specific to each cell type

and delivery method must be considered in detail, overall the safety profile of current approaches is generally favorable. For example, although none of the trials to date has been designed or empowered to examine hard clinical endpoints, it is encouraging that the largest randomized trial thus far—the REPAIR-AMI trial (15), which delivered unfractionated bone marrow cells (BMCs) to patients after a heart attack—as well as a recent meta-analysis of 50 similar trials enrolling 2625 patients (16) suggest that adverse clinical events may actually be less common in BMC-treated patients.

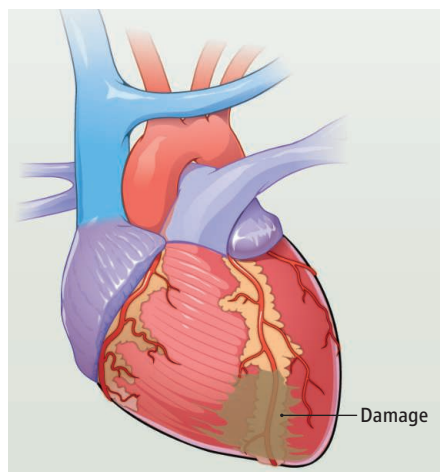
Another theme is that no clear winner has yet emerged from the multiple donor cell types being investigated. Autologous BMCs are by far the most common cells used to date but have yielded mixed results (17). Two recent trials report results with heart-derived donor cells. The SCPIO trial targeted patients with cardiac dysfunction undergoing bypass surgery for subsequent delivery of c-kit-positive cells derived from heart tissue harvested at surgery (18). In interim analyses, cardiac function was substantially better at 4 months in the 14 cell-treated patients available for comparison to seven control patients (18). In CADUCEUS, autologous cells derived from cardiospheres grown from cardiac biopsies (CDCs) were delivered to patients randomized after myocardial infarction to receive CDCs or usual care (19). In this trial, although overall heart function was not significantly improved by cell treatment, scar (determined by magnetic resonance imag-

The heart has greater potential for repair than once appreciated—whether this can be exploited clinically remains an open question.

ing) was reduced at 6 and 12 months in the 17 CDC-treated patients but unchanged in the eight control patients. Although both of these studies break new conceptual ground, it is still too early to know how these approaches will hold up in larger studies or impact clinical outcomes, and whether heart-derived cells will have demonstrable advantages over other cell types.

A third theme to emerge is that while donor cells have often been selected for their apparent ability to form new cardiomyocytes, the limited clinical data available suggest that relatively few of the donor cells may remain in the heart (20). Other benefits of the cells or molecules delivered with them could include enhanced angiogenesis, cardiomyocyte survival, or endogenous regeneration. The success or failure of cardiovascular cell therapy will ultimately depend on its ability to improve clinical outcomes whatever the mechanisms, and advocates argue that the donor cells may provide a particularly potent mixture of salutary effects. However, the complex and sometimes heterogeneous cell preparations being infused make standardization and reconciling discrepant results particularly challenging. It seems likely that identification and purification of the essential cellular and molecular components mediating any observed benefits will ultimately provide the most effective, safe, and consistent approach.

Recent work has begun to elucidate the settings and pathways regulating cardiomyocyte regeneration in animal models. Porrello *et al.* demonstrated a remarkable though transient regenerative capacity of the neonatal murine heart (14), and related studies have begun to define the signaling mechanisms leading to withdrawal of cardiomyocytes from the cell cycle (21). The Hippo pathway is a potent negative regulator of Wnt signaling and cardiomyocyte proliferation (22), which also intersects via Yap with insulin growth factor I (IGF-I) signaling (23). How effectively these pathways can be coopted to promote regeneration after injury is of great interest. Individual pathways may also have multiple effects. On page 1599 of this issue, Huang *et al.* (24) demonstrate that C/EBP inhibition, previously implicated in exercise-induced cardiac growth and possible cardiomyogenesis (25), also reduces ischemic injury by mitigating inflammation. In addition to endogenous path-



A damaged heart.

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ways, reprogramming resident nonprogenitor cells such as fibroblasts through gene delivery has generated contractile cardiomyocyte-like cells (26, 27) that mitigate scar formation and improve function after heart attacks in mice (28). These promising developments have yet to be translated clinically but could provide a path to cardiac repair that obviates the need for exogenous cells.

We are still relatively early in the development of new approaches to cardiovascular disease. It will be some time before we know the conclusion of what will likely be a long and challenging road ahead. Almost as challenging is conveying to patients and policymakers an appropriate perspective that balances unmitigated enthusiasm for the scientific discoveries, cautious optimism for the

broader implications, and humble acknowledgment that though even the most appealing ideas may fail, there is only one way to find out.

References and Notes

1. V. L. Roger *et al.*, *Circulation* **125**, e2 (2012).
2. CDC (2012), www.cdc.gov/dhdds/data_statistics/factsheets/docs/fs_heart_failure.pdf
3. C. J. DeFrances, M. N. Podgornik, *Adv. Data* **371**, 1 (2006).
4. T. E. Owan *et al.*, *N. Engl. J. Med.* **355**, 251 (2006).
5. R. S. Bhatia *et al.*, *N. Engl. J. Med.* **355**, 260 (2006).
6. J. Narula *et al.*, *N. Engl. J. Med.* **335**, 1182 (1996).
7. G. Olivetti *et al.*, *N. Engl. J. Med.* **336**, 1131 (1997).
8. A. P. Beltrami *et al.*, *Cell* **114**, 763 (2003).
9. K. Bersell, S. Arab, B. Haring, B. Kühn, *Cell* **138**, 257 (2009).
10. P. C. H. Hsieh *et al.*, *Nat. Med.* **13**, 970 (2007).
11. O. Bergmann *et al.*, *Science* **324**, 98 (2009).
12. J. Kajstura *et al.*, *Circ. Res.* **107**, 305 (2010).
13. K. Kikuchi *et al.*, *Nature* **464**, 601 (2010).
14. E. R. Porrello *et al.*, *Science* **331**, 1078 (2011).
15. V. Schächinger *et al.*, *N. Engl. J. Med.* **355**, 1210 (2006).
16. V. Jeevanantham *et al.*, *Circulation* **126**, 551 (2012).
17. A. Rosenzweig, *N. Engl. J. Med.* **355**, 1274 (2006).
18. R. Bolli *et al.*, *Lancet* **378**, 1847 (2011).
19. R. R. Makkar *et al.*, *Lancet* **379**, 895 (2012).
20. M. Hofmann *et al.*, *Circulation* **111**, 2198 (2005).
21. E. R. Porrello *et al.*, *Circ. Res.* **109**, 670 (2011).
22. T. Heallen *et al.*, *Science* **332**, 458 (2011).
23. M. Xin *et al.*, *Sci. Signal.* **4**, ra70 (2011).
24. G. N. Huang *et al.*, *Science* **338**, 1599 (2012); 10.1126/science.1229765.
25. P. Boström *et al.*, *Cell* **143**, 1072 (2010).
26. M. Ieda *et al.*, *Cell* **142**, 375 (2010).
27. L. Qian *et al.*, *Nature* **485**, 593 (2012).
28. K. Song *et al.*, *Nature* **485**, 599 (2012).

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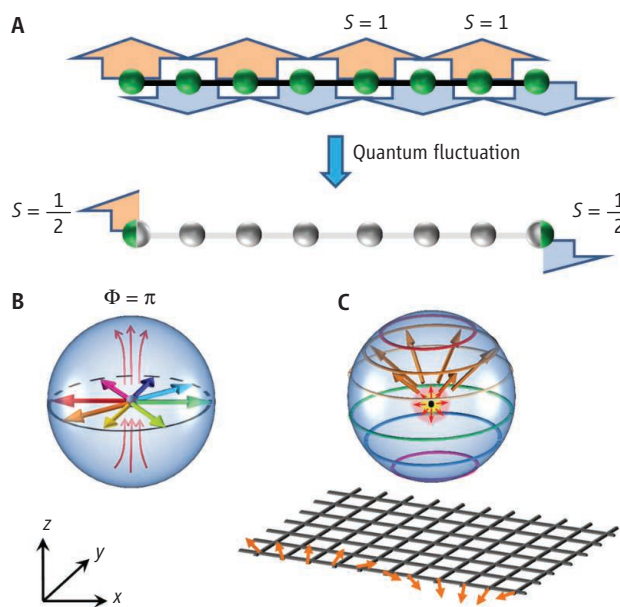
PHYSICS

Symmetry Meets Topology

Xiao-Liang Qi

Electrons possess the quantum mechanical property known as spin, often viewed in simple terms as the electron rotating. Quantum mechanics tells us that the amplitude of electron spin is restricted to integer multiples of $\frac{1}{2}$ units of the Planck constant, which means that the electron wave function changes sign (from positive to negative and vice versa) after a full rotation of 2π radians. This sign change determines the fermionic statistics of electrons, and similarly for all other leptons and quarks, without which no matter would be able to form. A natural question is whether the half spin (or spin- $\frac{1}{2}$ in usual notation) of the electron has to be a fundamental property. In other words, if the universe were populated by particles that had only integer spin, which do not change sign upon full rotation, would it be possible to combine them and make spin- $\frac{1}{2}$ particles? Interestingly, the answer to this question, and its generalization, is deeply connected to a seemingly unrelated subject in condensed matter physics—the classification of topological states of matter, the topic addressed by Chen *et al.* (1) on page 1604 of this issue.

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Spin- $\frac{1}{2}$ particles can actually emerge from spin integer ones (see the figure, panel A). In a chain of spin-1 particles with anti-ferromagnetic interaction, spins prefer to be antiparallel to each other, so that the net spin vanishes everywhere along the chain. According to the Haldane conjecture (2), the ground state of such a spin chain is a gapped state, that is, a state that is rigid in the sense that any change to it has to cost finite energy. However, the cancellation is incomplete at the boundary. In the bulk, each upspin is

A unified theory may describe both the persistent spin of electrons and the design of novel materials.

Spin and cohomology. (A) A spin-1 Haldane chain with all spins canceled in the bulk and spin- $\frac{1}{2}$ edge states at the ends. (B) A spin in the x-y plane rotating around the z axis. The sign change obtained by spin- $\frac{1}{2}$ can be understood as an Aharonov-Bohm phase induced by a half quanta of magnetic flux threaded in the ring. (C) A 2D SPT. The edge spin end points connect into a string, and the time evolution of the string can enclose a monopole point, leading to an Aharonov-Bohm phase.

canceled by the two downspins at its neighbors, but the spin at the end has only one neighbor. This is the reason for the spin- $\frac{1}{2}$ remaining at each end. During a full rotation, each spin- $\frac{1}{2}$ particle acquires a minus sign, which cancel each other as is required by the property of the underlying integer spin particles. In the presence of spin-rotation symmetry, the spin- $\frac{1}{2}$ at each end is robust to any local perturbation, because local perturbations can only add or remove integer spin. Such robust properties under arbitrary perturbation are called topological properties, just like the linking between two rings, or the difference between an annulus and a Möbius belt. Correspondingly, such a spin chain (known as the Haldane chain) is an example of a symmetry-protected topological (SPT) state, which is rigid in the bulk but is dis-

tinguished from a “boring” gapped state by the fractionalized spin- $\frac{1}{2}$ at each end. Other known examples of such SPT states include the time-reversal invariant topological insulators and superconductors discovered in recent years (3–5).

Chen *et al.* propose a generic classification scheme of SPT states in general dimensions by using the mathematical tool of group cohomology. Heuristically, cohomology studies how many ways exist to thread magnetic fluxes into a manifold. To see its relation to spin fractionalization, consider a spin vector rotating in the x - y plane (see the figure, panel B). If we think of the tip of the spin vector as a charged particle, a full rotation of the spin around the z axis corresponds to the particle moving around the equator ring by a full cycle. If we then thread a half quanta of magnetic flux through the ring, the wavefunction changes sign as the particle goes around the ring, known as the Aharonov-Bohm effect. Possible values of the fluxes, that is, the cohomology, determine the possible distinct topological states. In generic cases, the cohomology should be that of the symmetry group (I) instead of the trajectory of the spin, and the discussion above should only be taken as a heuristic picture.

The group cohomology approach has been previously applied to classifying one-dimensional (1D) topological states (6–8). The most important progress of Chen *et al.*

is the generalization to higher dimensions, which can also be understood in the Aharonov-Bohm effect picture. For example, for a 2D lattice model (see the figure, panel C), there is a line of spins along its edge. Therefore, the end points of these spins form a string in space, instead of a single point in the case of the boundary of one dimension. The time evolution of the string is a surface, which is the analog of the particle orbit in the 1D case. One can then define a flux threaded into the surface, which is a monopole point instead of a flux line. If the string moves around the monopole point, it obtains an Aharonov-Bohm phase that fractionalizes the string. Such magnetic fluxes observed by strings, or higher-dimensional membranes, are characterized by higher cohomology groups of the symmetry group. Chen *et al.* describe the topological states classified by such group cohomology in generic dimensions by presenting exact solvable prototype lattice models for each class, with explicitly defined actions and ground-state wave functions.

Chen *et al.*'s approach is powerful because it describes a large class of new topological states in general dimensions, which is a major expansion of our knowledge on SPT states in interacting many-body systems. Many interesting questions follow: Because the prototype models proposed are discrete lattice models, is there a continuous field the-

ory description for each topological class? Because this approach has been recently generalized to fermion systems (9), can it include the free fermion topological insulators and superconductors? And what materials can realize the proposed models?

From the quantum Hall effect to topological insulators, the interplay between symmetry and topology keeps providing us pleasant surprises. The understanding of SPT states suggests that what we have learned so far is only the tip of the iceberg. Besides predicting novel materials, better understanding of symmetry and topology may also shed new light on the understanding of some of the deepest mysteries of our universe, such as the electron spin.

References and Notes

1. X. Chen, Z.-C. Gu, Z.-X. Liu, X.-G. Wen, *Science* **338**, 1604 (2012).
2. F. D. M. Haldane, *Phys. Lett. A* **93**, 464 (1983).
3. M. Z. Hasan, C. L. Kane, *Rev. Mod. Phys.* **82**, 3045 (2010).
4. J. E. Moore, *Nature* **464**, 194 (2010).
5. X.-L. Qi, S.-C. Zhang, *Rev. Mod. Phys.* **83**, 1057 (2011).
6. L. Fidkowski, A. Kitaev, *Phys. Rev. B* **83**, 075103 (2011).
7. A. M. Turner, F. Pollmann, E. Berg, *Phys. Rev. B* **83**, 075102 (2011).
8. X. Chen, Z.-C. Gu, X.-G. Wen, *Phys. Rev. B* **83**, 035107 (2011).
9. Z.-C. Gu, X.-G. Wen, arXiv:1201.2648 (2012).

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GEOCHEMISTRY

Modeling the Formation of Porphyry-Copper Ores

Steven E. Ingebritsen

Porphyry-copper ore systems, the source of much of the world's copper and molybdenum, form when metal-bearing fluids are expelled from shallow, degassing magmas. On page 1613 of this issue, Weis *et al.* (1) demonstrate that self-organizing processes focus metal deposition. Specifically, their simulation studies indicate that ores develop as consequences of dynamic variations in rock permeability driven by injection of volatile species from rising magmas. Scenarios with a static permeability structure could not reproduce key field observations, whereas dynamic perme-

ability responses to magmatic-fluid injection localized a metal-precipitation front where enrichment by a factor of 10^3 could be achieved [for an overview of their numerical-simulation model CSMP++, see (2)].

Dynamic variations in crustal permeability are likely a key to the genesis of many ore deposits, and are also of great interest in the context of enhanced gas and oil production (“fracking”), enhanced geothermal systems (EGSs), geologic carbon sequestration, and both natural and induced seismicity. The permeability of Earth's crust largely governs important geologic processes such as the advective transport of heat and solutes and the generation of elevated fluid pressures by processes such as physical compaction and

Dynamic changes in the permeability of Earth's crust are needed to account for the formation of porphyry-copper ores from magmatic fluids.

mineral dehydration. For an isotropic material, permeability k is defined by Darcy's law, which relates the fluid discharge per unit area q to the gradient of hydraulic head h as $q = (kg\rho/\mu) \nabla h$, where ρ is fluid density, μ is fluid viscosity, and g is gravity. Although the permeabilities of common geologic media vary by approximately 16 orders of magnitude, k can sometimes be characterized at the crustal scale in a manner that provides useful insight (see the figure) (3–5).

Hydrogeologists and petroleum engineers traditionally treat permeability as a static material property that exerts control on fluid flow, but many economic geologists, geophysicists, and metamorphic petrologists have long recognized that permeability

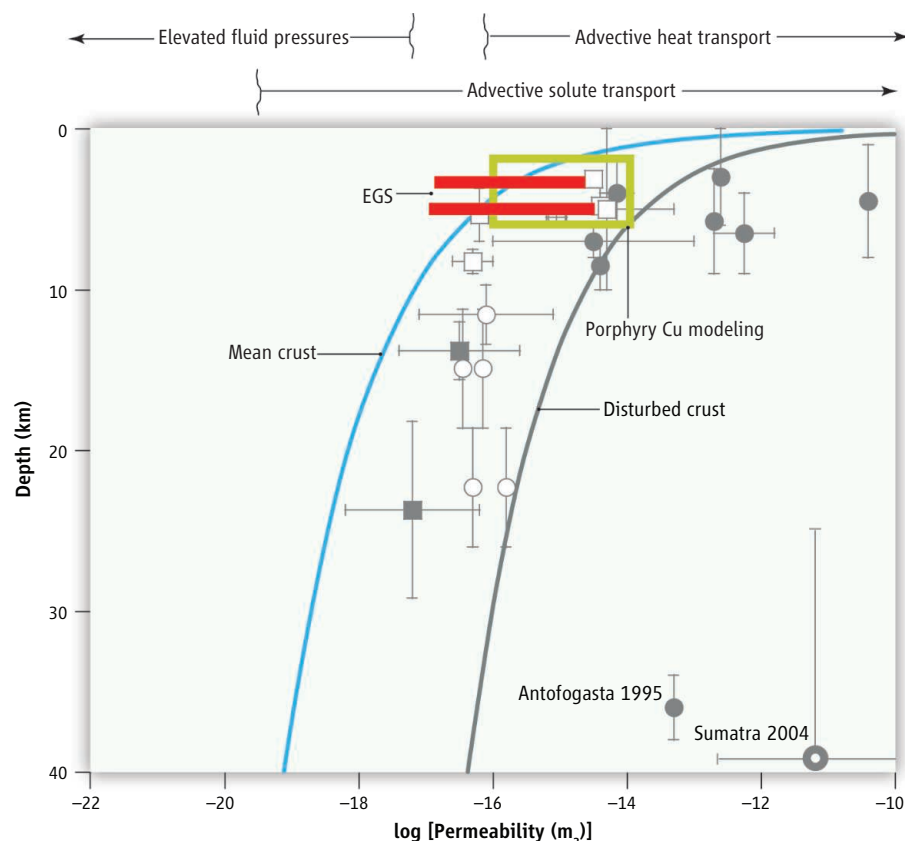
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How permeable is the crust? Estimates of “mean crust” permeability k shown here are based on hydro-thermal modeling and the progress of metamorphic reactions (4), but on geologically short time scales, k may greatly exceed these values (5). The power-law fit to these high- k data [not including the recently published Sumatra datum (11)] is labeled “disturbed crust.” The evidence includes rapid migration of seismic hypocenters (solid circles), enhanced rates of metamorphic reaction in major fault or shear zones (open circles), recent studies suggesting much more rapid metamorphism than had been canonically assumed (solid squares), and anthropogenically induced seismicity (open squares); error bars depict the full permissible range for a plotted locality and are not Gaussian errors. Red lines indicate k values before and after EGS reservoir stimulation at Soultz (upper line) (12) and Basel (lower line) (13), and the green rectangle is the k -depth range invoked in modeling by Weis *et al.* of the formation of porphyry-copper ores. Arrows above the graph indicate approximate ranges of k over which certain geologically important processes are likely.

can respond to dewatering and fluid production [see, for example, (5, 6) and references therein]. This dynamic view of crustal permeability is consistent with indications that during prograde metamorphism (in which volatiles are lost), fluid pressure is nearly in balance with the lithostatic load of the overburden (7); sufficiently overpressured fluids cannot be contained in the crust and create the permeability necessary to escape. The permeability of the brittle upper crust may also be dynamically self-adjusting (8, 9).

Weis *et al.* implemented these concepts by using permeability ranges similar to those actually observed in EGSs at similar depths (see the figure). EGS technology aims to enhance heat extraction by fracturing rock with insufficient natural permeability. The bulk permeability of $\sim 1 \text{ km}^3$ of rock increased by a factor of 100 or more in EGS experiments that injected water at rates of tens of kilograms per second, rates similar to those invoked by Weis *et al.* for volatile injection.

A debatable assumption by Weis *et al.* is that permeability is negligibly low between 350° and 400°C , a temperature range associated with the brittle-to-ductile transition (BDT). The argument that a certain permeability is required for volatile release applies both above and below the BDT, and “mean crust” permeabilities on the order of 10^{-19} to 10^{-18} m^2 have been inferred to midcrustal depths on that basis (see the figure). The BDT depends on mineralogy and strain rate as well as temperature [e.g., (10)], and in the Weis *et al.* study, it occurs at shallow depths (a few kilometers) because the crust is locally heated by igneous intrusion (typically the



BDT occurs at 10 to 15 km depth). The most extreme examples of inferred high permeabilities at great depth come from subduction zones, which are cooled by subducting crust. For instance, a recent reanalysis of the 2004 magnitude 9.2 Sumatra-Andaman earthquake attributes certain aftershock sequences to the migration of aqueous fluids along splay faults, and infers permeabilities on the order of 10^{-12} to 10^{-10} m^2 at depths of 25 to 55 km (11). These values are comparable to permeabilities of young, unaltered volcanic rocks in the near surface.

The figure compares data that represent the mean permeability of the tectonically active continental crust (4), continental crust disturbed by various transient processes (5), selected EGS experience (12, 13), and the porphyry-copper simulations. The higher permeability values must be ephemeral in the context of geologic time; for instance, such high values would imply that heat transport in the continental crust is dominated by advection rather than conduction (see the figure), which is demonstrably not the case. However, as in the porphyry-copper study, ephemeral episodes of higher permeability can lead to localized heat and mass transport and may be linked to certain kinds of seismicity.

The innovative study by Weis *et al.* represents a happy marriage of the Earth science

disciplines of hydrogeology and economic geology (the latter being that branch of the Earth sciences concerned with the genesis of ore deposits). The number of economic geologists has declined to $<2\%$ of total U.S. geoscience faculty (14); for practical and strategic issues related to mineral supply, a resurgence of this discipline is needed.

References and Notes

1. P. Weis, T. Driesner, C. A. Heinrich, *Science* **338**, 1613 (2012); 10.1126/science.1225009.
2. <http://csmpe.ese.imperial.ac.uk/wiki>
3. T. Gleeson *et al.*, *Geophys. Res. Lett.* **38**, L02401 (2011).
4. C. E. Manning, S. E. Ingebritsen, *Rev. Geophys.* **37**, 127 (1999).
5. S. E. Ingebritsen, C. E. Manning, *Geofluids* **10**, 193 (2010).
6. M. Manga *et al.*, *Rev. Geophys.* **50**, RG2004 (2012).
7. W. S. Fyfe, N. J. Price, A. B. Thompson, *Fluids in the Earth's Crust* (Elsevier Scientific, New York, 1978).
8. J. Townend, M. D. Zoback, *Geology* **28**, 399 (2000).
9. S. A. Rojstaczer, S. E. Ingebritsen, D. O. Hayba, *Geofluids* **8**, 128 (2008).
10. F. Simpson, *Geofluids* **1**, 123 (2001).
11. F. Waldhauser, D. P. Schaff, T. Diehl, E. R. Engdahl, *Geology* **40**, 243 (2012).
12. K. F. Evans, A. Genter, J. Sausse, *J. Geophys. Res.* **110**, B04203 (2005).
13. M. O. Häring, U. Schanz, F. Ladner, B. C. Dyer, *Geothermics* **37**, 469 (2008).
14. M. Hitzman, J. Dilles, M. Barton, M. Bolland, *GSA Today* **19**, 26 (2009).

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RETROSPECTIVE

Keith Campbell (1954–2012)

Ian Wilmut

Keith Campbell, who died on 5 October at the age of 58 in the Derbyshire region of England, was instrumental in the birth of Dolly the sheep, in 1996 the first animal to be cloned from an adult cell. Her birth overturned a long-standing belief that the mechanisms that regulate the formation of all of the different tissues of an adult are so complex and rigidly fixed that it would not be possible to change their fate. This unexpected result prompted later research by Shinya Yamanaka and James Thomson, who developed methods for the production of pluripotent cells directly from somatic cells, an advance that will have profound effects in research and, ultimately, in cell therapy.

Keith Henry Stockman Campbell was born in Birmingham, England, on 23 May 1954. The family moved to Perth in Scotland when he was 3 years old before returning to Birmingham 5 years later. He was educated at King Edward VI Grammar School for boys and then trained at Selly Oak Hospital as a medical laboratory technologist specializing in medical microbiology.

He obtained a B.Sc. in microbiology in 1978 at Queen Elizabeth College, London, and it was during these studies that Keith first became interested in mechanisms that regulate the growth and division of cells: the cell cycle. After brief temporary positions, first as a chief medical laboratory technologist in southern Yemen and then in a program to eradicate Dutch Elm disease from parts of southern England, he joined the cytogenetics group of Nutan Bishun at the Marie Curie Research Institute in Surrey. The Marie Curie Foundation supports basic research in the underlying causes and mechanisms of cancer, and his interests in the regulation of cellular growth and differentiation developed further. In 1983, Keith was awarded a Marie Curie Research Scholarship and moved to the University of Sussex as a postgraduate student. There, he studied the cytoplasmic control of nuclear events during the development of amphibian oocytes, in early embryos, and during cell growth and cell division in yeast. He was



awarded a D.Phil. in 1986 for his thesis on aspects of cell cycle control in both of these model organisms.

On completion of these postgraduate studies, Keith moved to Scotland where, after two postdoctoral positions, he joined the Roslin Institute in Edinburgh in 1991, where we worked together. His research experience on cell cycle control was a perfect fit for the cloning project to which he was appointed. When combined with our extensive experience of mammalian embryo recovery, transfer, and micromanipulation, it gave us all of the skills that were needed to complete the project. During the following 6 years, Keith applied his understanding of the cell cycle to the production of mammalian embryos by a process called nuclear transfer. This involves removing DNA from an oocyte and then injecting it with the nucleus of another cell (containing the DNA to be cloned). If the oocyte divides and replicates the new DNA, the cloned cells (in the form of a blastocyst) can be placed in the uterus of a female animal, and a cloned organism can result. In 1995, this research led to the birth of Megan and Morag, two Welsh mountain lambs, the first mammals to be cloned from cultured, differentiated cells. In 1996, these experiments were repeated and extended, resulting in the birth of Dolly, the first animal to be cloned from an adult-derived somatic cell. These successes depended on the protocols that Keith established to restore normal cell cycles after transfer of the nucleus.

The aims of the cloning studies were twofold: to provide a means for the precise genetic modification of farm animal spe-

A cell biologist interested in the cell division cycle worked on Dolly the sheep, the first cloned mammal.

cies, and to understand the basic mechanisms underlying cellular differentiation. In a collaborative project with PPL Therapeutics, the biopharmaceutical company that had been spun off from Roslin Institute, the cloning procedure was used to introduce a genetic change. Polly was the first transgenic mammal (sheep) to be produced by nuclear transfer from a cell line genetically modified in culture. Polly contained the human gene encoding Factor IX, a protein involved in preventing hemophilia. This was the first step in producing animals whose tissues or milk might contain large quantities of proteins for therapeutic use.

Initially a consultant for PPL Therapeutics, Keith left the Roslin Institute in 1997 to become head of embryology for the company. While he was at PPL, the cloning procedure led to the birth of cloned and genetically modified sheep, pigs, and cattle, including the first gene-targeted lambs (Cupid and Diana) in 1999, and the first piglets cloned from somatic cells in 2000.

In 1999, Keith left PPL Therapeutics to become professor of animal development at the University of Nottingham. There, he continued his research into the basic mechanisms underlying cell differentiation and development to improve and understand the cloning process and to develop reproductive technologies in farm animals to enhance breeding and maintain food security.

Keith served as an editorial board member for a number of journals and was on scientific advisory boards of a number of companies and academic organizations. In 2008, he and I were awarded the Shaw Prize for Medicine and Life Sciences jointly with Shinya Yamanaka.

Keith Campbell was an engaging and friendly person who was always passionate about whatever he was doing, no matter whether it was research, mountain biking around the Scottish countryside, or cooking. Much of his research was presented and discussed at the annual meeting of the International Embryo Transfer Society, where he will long be remembered as an enthusiastic participant in discussions that lasted late into the night. Always cheerful and very friendly, with a strong distaste for bureaucracy, he will be sorely missed but not forgotten.

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How We Got Here: An Inquiry-Based Activity About Human Evolution

Rebecca M. Price

How your students see the face of a baby chimpanzee, and they will be startled and amazed by how human she is (see the photo). The image intrigues and primes students for a scientific inquiry cycle in which they will engage with, explore, explain, elaborate, and evaluate data [the 5e inquiry cycle (1)]. Through inquiry, they will reconsider one of the most common misunderstandings about evolution (2), that humans evolved from chimpanzees. The class is ready for a conceptual change (3) based on evidence the students will gather.

Students spend the majority of class time testing the hypothesis that the evolution of skull shape within the human lineage took place largely by truncating the development of a chimpanzee-like ancestor. They work collaboratively, mimicking the creativity in scientific practice (4), by collecting data from the casts of skulls of fetal, infant, juvenile, and adult chimpanzees, as well as adult skulls of *Homo sapiens*, *Homo erectus*, *Australopithecus afarensis*, and *Ardipithecus ramidus*. They recognize that developmental changes in shape are a source of heritable variation on which evolutionary processes can act.

This learning module is flexible; one can choose learning goals that are appropriate to the course content (see the table). Elsewhere, I described one approach that focused on learning about evolution (5). Here, the approach emphasizes goals for learning about the nature and process of science through three cycles of inquiry (see the chart).

Step 1. The instructor shows students a picture of a baby chimpanzee (such as the one in the photo). The students spend 1 minute writing (6) about why humans and chimpanzees look so similar; this brainstorming sets the stage for the instructor to introduce the testable hypothesis—that our evolutionary ancestor looked like a chimpanzee.

Step 2. Pairs of students observe casts of chimpanzee skulls and identify several characteristics, such as how protuberant the eye



How We Got Here, an IBI prize-winning module, utilizes iterative cycles of inquiry to help students learn about evolution.

Chimpanzee. The startling similarities between baby chimpanzees and humans engage students in the scientific process.

Step 5. Pairs of students answer questions provided by the instructor (5). Whole-class discussion helps clarify confusing points.

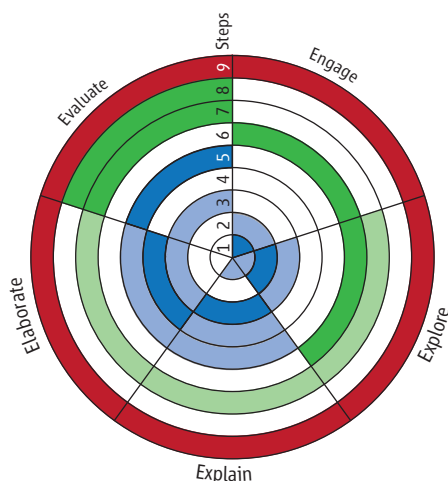
Step 6. Students begin a second inquiry cycle (see the chart) when they analyze *Ardipithecus ramidus*, also known as “Ardi.” This second cycle models a paradigm shift from the original hypothesis to a more sophisticated one. The original hypothesis that the hominin ancestor looked like a chimpanzee was widely accepted by experts until recently (8). Students focus on skull measurements, but consider other anatomical features. Ardi demonstrates that our species retains a host of primitive traits and that chimpanzees evolved a number of derived traits (8). From their analysis, they infer that Ardi appeared much later, evolutionarily, than she actually did. The paradigm shift begins when I tell the students Ardi’s true age, and we work, as a class, to reconstruct the evolutionary relationships of the species in the activity.

Step 7. Additional questions (5) provided by the instructor complete the paradigm shift, and students revise their hypotheses. By answering the questions in small groups and then discussing them with the whole class, students recognize that chimpanzees undergo more shape change before reaching sexual maturity than Ardi did, whereas humans undergo less shape change by the time we reach maturity.

brow ridges are, or how long a skull is, that describe the changes that occur during development. Having students identify the characteristics they will study involves them in the process of experimental design. They may choose characteristics that the instructor has not considered, such as the curvature of the back of the skull. As with authentic research (7), their results are unpredictable.

Step 3. Students illustrate what the hypothesis predicts, drawing the skull shapes that they think a new species might have. We discuss how the characteristics that they identified provide evidence to test their hypothesis.

Step 4. Pairs of students choose two characteristics to quantify shape and one measurement, such as skull width, to standardize the others. They evaluate these traits in all the skulls except that of *Ardipithecus ramidus* and graph their results [e.g., (5)].



Primary, Secondary foci
 Cycle 1: Chimpanzees
 Cycle 2: Ardi
 Cycle 3: Piltown Man

Three different inquiry cycles. The steps targeting the 5e inquiry cycle (dark colors) are shown; they may also include secondary foci (light colors). In the first cycle (steps 1 to 5, blue), students test the hypothesis that humans evolved from the truncated development of a chimpanzee-like ancestor by identifying characteristics, graphing data, and interpreting results. In the second cycle (steps 6 to 8, green), students revise their conclusion from cycle 1 after considering *Ardipithecus ramidus*. Students’ understanding of inquiry is more sophisticated and they navigate the cycle with less scaffolding. In the third cycle (step 9, red), students test the hypothesis that Piltown man is ancestral to humans. At this stage, they understand the inquiry cycle well enough that they need little guidance from the instructor.

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 *IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.

Step 8. Students return to step 1 in pairs to reevaluate their original ideas and to consider how the characteristics they chose affected their outcome. Then, the whole class discusses the results for each pair of characteristics. Some combinations support the hypothesis that the hominin ancestor looked like a chimpanzee; others cause it to be rejected. For example, the location of Ardi's foramen magnum is more similar to a chimpanzee's, whereas her canines are more like ours (8). When we seek consensus among all the students' data, we always conclude that we should reject our initial hypothesis.

Step 9. In the third inquiry cycle (see the chart), students test the hypothesis that Pilt-down man is ancestral to hominins. They infer that the cranium is *Homo sapiens*, rejecting the hypothesis. This step brings ethics, scandal, and the history of science into the conversation.

Steps 1 to 9 are framed by testing a hypothesis about the morphology of the human ancestor. Students ultimately reject the first hypothesis and formulate an alternative: that the ancestor shared human and chimpanzee characteristics. Framing this activity around hypothesis testing forestalls debate about whether evolution is occurring, focusing instead on how scientific conclusions are formulated. By acknowledging that science is one of many ways of knowing (9), I emphasize that some students may personally prioritize faith over science, but that I expect them to recognize the scientific reasoning supporting evolution and the lack of scientific evidence rejecting it.

Experts read deep meaning into symbols within their area of expertise (3). Our students do not know how to gain an overview of the scientific process from activities that are written without ambiguity, even though

Course emphasis

Evolution

Evolution and development

Scientific Process

Quantitative literacy

Learning goals

- Use evidence to explain the inaccuracies in the statement "humans evolved from chimpanzees."
- Practice building and interpreting phylogenetic trees.
- Recognize that evolutionary change results in a mosaic of primitive and specialized characteristics within each species.
- Explore the false dichotomy between macroevolution and microevolution.
- Employ the data presented in this lab to promote tree-thinking and dismantle the misconception that evolution embodies linear progress.
- Recognize that developmental changes in shape are a source of heritable variation upon which evolutionary processes can act.
- Introduce students to the morphological perspective of evo-devo.
- Analyze data from and summarize the history of one of the most famous cases of scientific fraud.
- Evaluate the process of a paradigm shift.
- Practice scientific inquiry by collecting data, constructing, and interpreting graphs, and using results and new data to test and revise a hypothesis.
- Devise different ways to graph and tabulate data.
- Evaluate why standardizing data aids and inhibits analysis.

A modifiable learning module. Different goals can be emphasized to suit course content.

experts recognize that recipe-based labs are symbolic representations of science. Inquiry-based learning makes explicit the dynamism and creativity in science (1).

The development of this activity mirrors my development as an educator. When I used a recipe-based approach, I dictated which characteristics students should measure, ensuring that everyone achieved the same results. But after researching effective pedagogy and gaining experience, I realized that my students were not engaged in the scientific process. Moreover, it was difficult to distinguish between students who achieved a deep understanding of the material and those who had memorized the answers they knew I wanted. I learned about inquiry [e.g.,

(1)], recognizing it as a formal pedagogical approach and then reframed this activity. The most transformative insight was learning to emphasize engagement. Most students do not share our scientific passions and are bored unless they see a reason for doing an activity. But an engaging activity inspires passion. The intrigue borne from looking into the face of a baby chimpanzee opens their eyes to discovery.

References and Notes

1. R. W. Bybee, *Achieving Scientific Literacy: From Purposes to Practices* (Heinemann, Portsmouth, NH, 1997).
2. W. E. Meikle, E. C. Scott, *Evol. Educ. Outreach* **3**, 573 (2010).
3. J. D. Bransford, A. L. Brown, R. R. Cocking, *How People Learn: Brain, Mind, Experience, and School, Expanded Edition* (National Academy Press, Washington, DC, 2000).
4. R. L. DeHaan, *Science* **334**, 1499 (2011).
5. R. M. Price, *Am. Biol. Teach.* **74**, 106 (2012).
6. J. Handelsman, S. Miller, C. Pfund, *Scientific Teaching* (Freeman, New York, 2007).
7. K. K. Karukstis, T. E. Elgren, Eds., *Developing and Sustaining a Research-Supportive Curriculum: A Compendium of Successful Practices* (Council on Undergraduate Research, Washington, DC, 2007).
8. C. O. Lovejoy, *Science* **326**, 74 (2009).
9. E. C. Scott, *Evolution vs. Creationism: An Introduction* (Univ. of California Press, Berkeley, CA, 2004).
10. This activity was inspired by another activity written by D. Jablonski and M. Foote. C. Tzou introduced me to the 5e Inquiry Cycle. Thanks to B. Burgett, G. Kochhar-Lindgren, and M. Servetnick for their support, to S. Rosenthal, and to the many students who have participated in this activity. M. Groom, J. Peters, and editors at *Science* offered valuable advice for improving this piece.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6114/1554/DC1

About the author

When **Becca Price** was a little girl, a museum exhibit about the famous australopithecine Lucy inspired her to become an evolutionary biologist. She completed a Ph.D. at The University of Chicago, where her research focused on how and why sea shell shapes have changed through deep time. She participated in the Seeding Postdoctoral Innovators in Research and Education (SPIRE) Postdoctoral Fellowship Program at the University of North Carolina at Chapel Hill, which introduced her to the scholarship of teaching and learning. Now an Assistant Professor at the University of Washington, Bothell, Becca's primary research focus is studying effective methods for helping students understand evolution. She is coprincipal investigator of a working group at the National Evolutionary Synthesis Center that is developing a series of concept inventories to measure students' understanding of different aspects of evolution. In her teaching practice, she uses interdisciplinary strategies that help students relate classroom knowledge to the real world.





SCIENCE AND SOCIETY

Traumatic Brain Injury: New Insight, But Treatment Remains Elusive

He was a veteran professional football player, in his mid-60s when he died, and a paper-thin cross section of his brain tissue taken at the autopsy appears visibly shrunken and atrophied. Perhaps that's no surprise in an older man who played in an era of more primitive equipment. But neuropathologist Ann McKee has other samples that show similar damage in soldiers and athletes who died far younger. One, a football player, was just 18.

Head injuries among soldiers and athletes are hardly a new discovery, but for decades they were a blind spot. A bullet hole would get you a ticket home; a broken arm or torn ligaments would force you from the game. But until recently, a warrior who sustained a concussion—who'd had his bell rung—would shake it off and then return quickly to the battlefield or the playing field.

Today, there's a growing alarm about the long-term dangers of traumatic brain injury (TBI). And in a discussion at AAAS, researchers described how injuries that show little abnormality on an MRI or CT scan can, years later, have debilitating effects ranging from irritability to rage and dementia. While scientists are learning much about the nature of these injuries, they said, therapies to protect or repair the brain are proving elusive.

The 23 October event, cosponsored by the Dana Foundation and AAAS, came at a critical time: Blast injuries, not bullets, are the dominant risk for soldiers deployed to

Iraq and Afghanistan. The National Football League is facing lawsuits from nearly 4000 ex-players, and several current or former players have committed suicide in recent years; safety concerns now extend to community pee-wee football programs. Taken together, those streams represent a seeming TBI epidemic.

Chronic traumatic encephalopathy (CTE) is neuron degeneration that occurs after mild but repetitive traumatic brain injury, more commonly called concussions and subconcussions. James L. Hancock endured three concussions playing football and rugby. Then, while he was a Navy doctor deployed with the Marines at an advance base in Afghanistan's Helmand province, his vehicle hit an improvised explosive device. The blast knocked him unconscious.

Captain Hancock told the AAAS audience that as he recovered, his sense of balance was compromised. He started to have migraines. "My emotions were absolutely flat," he added. "Sleep became a problem."

McKee, who is also a neurologist and director of the Neuropathology Service for the New England Veterans Administration Medical Centers, used images of stained brain tissue to illustrate features of CTE, including unusual deposits of the protein p-tau. Even "without the aid of a microscope...you can immediately see the abnormalities because they are so profound," she said. There is shrinkage and atrophy in the prefrontal cortex, the hippo-



campus, the amygdala, and other areas associated with learning, memory, judgment, and emotional control.

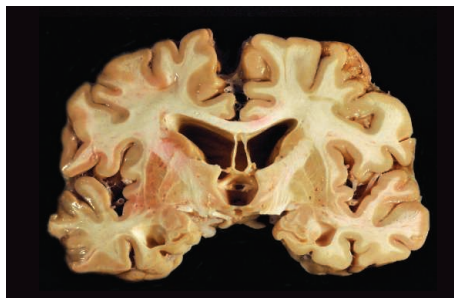
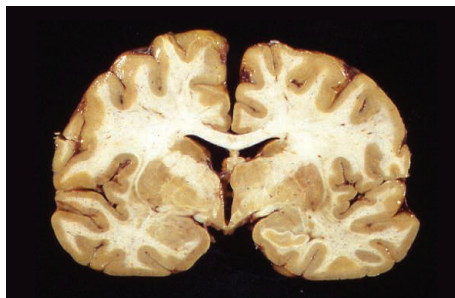
The first treatment guidelines for TBI were developed in the 1990s and were simply "to maintain the general physiology to support the brain," said Geoffrey Ling, a retired colonel and program manager at the Defense Advanced Research Projects Agency. Ling played a leading role in developing standard concussion treatment guidelines for the military and the Military Acute Concussion Evaluation tool, which enables frontline medics and junior officers to evaluate TBI.

The key medical reason to quickly identify TBI is "second-impact syndrome," Ling said. When a patient sustains a second head injury before fully recovered from the first, he explained, "it leads to an exaggerated response and has a 50% mortality rate."

While some drugs have shown neuroprotection in animals, Ling said, none have proven useful in humans. The standard of care for TBI today consists of letting the brain rest and providing symptom relief, primarily for pain, Hancock said, though he acknowledged that there's little evidence to back that practice.

Because so much remains unknown about the brain and the complex effects of TBI, "for us to try to grapple with all of the variables is impossible," McKee said. She believes that improved treatment will come through understanding the physical changes in the brain that occur at the microscopic and molecular levels when the brain is subject to trauma. And that understanding is only beginning to emerge.

—Bob Roehr and Edward W. Lempinen



Visible damage. Mild but repetitive brain injury can transform healthy brain tissue (left) into the atrophied and deteriorated tissue associated with chronic traumatic encephalopathy (right).

At Mexico Competition, Students Excel at Math and Diplomacy

GUANAJUATO, Mexico—Students from an elite group of 20 young mathematicians trained by AAAS made up the only U.S. team at the Olimpiada Mexicana de Matemáticas. They were up against 196 of the very best high school math students in Mexico, taking tests in a completely unfamiliar setting and trying to comprehend, in Spanish, complex problems that generally require more than an hour to solve.

After participating in AAAS's intensive program to identify and cultivate exceptional math talent among underrepresented minorities and the children of immigrants, the students were confident in their ability to compete. But their performance—and the quality of the international interaction—at the Olympiad reached unexpected heights. David Vargas, a high school senior from Roslyn, New York, came up with a solution to a problem that was so original that none of the other 199 contestants solved the problem in the same way.

Although the four U.S. students were not official competitors, their scores would have qualified for medals: silver for Vargas and Varun Mohan, a junior from Sunnyvale, California, and bronze for Chicago junior Emanuel Perez and Sohail Farhangi, a senior from Fairfax Station, Virginia.

The 11 to 16 November event followed 10 days of training in Washington, D.C., this

past summer, where the participants were selected from a pool of nearly 70 U.S. applicants. Four other students from the AAAS training participated in the Pan-African Mathematics Olympiad held in Tunisia in September.

The exceptionally gifted students are “not often celebrated for their talent and ability,” said AAAS Olympiad Program Director Florence Fasanelli, “but rather are left out and feel quite alone.” Program codirector Mark Saul, who also directs the Center for Mathematical Talent at New York University, said that when they do find a math club or team, it may have no other minority students or advisers.

“This event gives these students a security in the things they do, a confidence in themselves,” said José Antonio Gómez, OMM director and professor at the Universidad Autónoma de México. “They come to know themselves and learn that they can do things that they didn’t know they could do.”

The Mexican competition, which selects national contestants for the International Mathematics Olympiad, involved six problems solved over 2 days. During activities surrounding the testing, the students and their mentors, including University of Texas–San Antonio Professor Eduardo Dueñez, found time to make significant connections with their Mexican counterparts,



Math diplomacy. Emanuel Perez (center) and the AAAS team built significant connections with their Mexican peers during the Olympiad.

practicing an informal “math diplomacy” that added a valuable dimension to the trip.

After the testing ended, the U.S. and Mexican contestants traveled to a remote elementary school outside of Guanajuato, where they shared math-related puzzles, games, and demonstrations with students. The AAAS trainees said that the collegial atmosphere of the competition made it easy to form bonds with their Mexican peers. “It gives it more of a cooperative feeling, rather than a competitive one,” said Vargas. “It’s like we’re all working together.”

—Michaela Jarvis

14–18 FEBRUARY 2013

Annual Meeting Explores Beauty, Benefits of Science

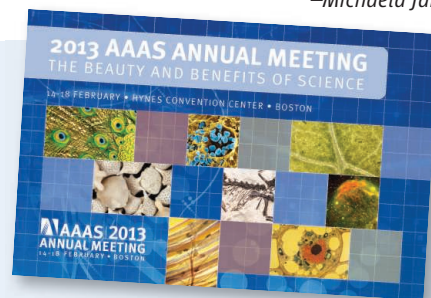
It’s a familiar pathway for many scientists: Their curiosity and drive to understand the world lead to broader benefits. The 2013 AAAS Annual Meeting, which convenes from 14 to 18 February in Boston, will focus on these deep and rich connections between the beauty of scientific knowledge and its often unexpected impact.

The 179th Annual Meeting will feature top scientists, engineers, educators, policy-makers, and science journalists from around the world. More than 150 sessions are scheduled under the theme “The Beauty and Benefits of Science,” including symposia in 14 research tracks, career development workshops, and popular public events such as Family Science Days.

Plenary speakers will include molecular biolo-

gist Cynthia Kenyon, whose work on roundworm genetics sparked an intensive new interest in the biology of aging, and Harvard astrophysicist Robert Kirshner, who guided two Nobel Prize winners to their discovery of an accelerating universe. Their research demonstrates “the rich and complicated connections between basic and applied research, and how they bring about both practical benefits and the beauty of pure understanding,” said AAAS President William H. Press in his letter of invitation.

The Meeting’s ambitious program includes topics such as teaching stroke victims to speak again, ensuring food safety in a global marketplace, and reaching a critical turning point in fusion energy research. Other presenters



will discuss the draft findings from the U.S. National Climate Assessment, the quiet successes of cybersecurity research, and key international teacher-scientist partnerships.

For registration and program information, see www.aaas.org/meetings. The site <http://news.aaas.org> will serve as a portal for Annual Meeting news from *ScienceNow*, *Science Update*, www.aaas.org, AAAS MemberCentral, and EurekAlert! The AAAS Facebook page and Twitter (@AAASmeetings; #AAASmtg) also will feature news from Boston.

The Higgs Boson

The standard model of particle physics describes how elementary particles and a set of forces between them lead to all matter and most higher interactions, thereby providing a basis for understanding much of physics and chemistry. A key prediction of the standard model—that the universe is pervaded by a field that conveys mass—was tested and confirmed this year with the discovery of a particle associated with that field: the Higgs boson. Two separate, complex detectors housed in the largest and most energetic particle accelerator, the Large Hadron Collider (LHC) at CERN near Geneva, Switzerland, identified the characteristic decay products of the Higgs, allowing reconstruction of its mass. These experiments and this discovery were made possible by decades of cutting-edge,

publicly supported science, engineering, and construction, and a long-term effort by the physics community worldwide.

In three articles in this issue, the researchers explain their results and the developments that led to the detection. The first presents the history of the search for the Higgs and the experimental approach, and summarizes the results. This is followed by a paper from each of the two detector teams describing their experiments and results in more detail. These papers aim to provide an accessible overview of the two research papers published this summer in *Physics Letters B*, which are the primary references. We hope that this package and the accompanying glossary give broad access to this discovery that is fundamental to understanding our universe.

Glossary

In the standard model (SM), several fundamental or elementary particles can interact to create all compound forms of matter. Several of these fundamental particles are also used to search for the Higgs boson, because they appear as its decay products. This glossary, developed by the lead authors of the three papers, provides an overview of some of the particles and also indicates some of the important abbreviations, notations, and terms used in particle physics and the papers.

Fundamental Particles

Leptons (ℓ): Fundamental particles that include the electron (e), muon (μ), and τ lepton (τ) that do not combine via the strong interaction.

Quarks: Fundamental particles that can combine via the strong interaction to form composite particles. One of the ways the Higgs boson decays is into a bottom quark and bottom anti-quark pair ($H \rightarrow b\bar{b}$).

Bosons: Bosons are particles with an integer spin quantum number (0, 1, 2, ...); they obey Bose-Einstein statistics, which allows multiple particles to occupy the same quantum state. They can be fundamental or composite particles. **Fundamental bosons** include the photon (γ), which carries the electromagnetic force; W or Z bosons, which carry the weak force; and gluons, which carry the strong force. All have a spin of 1. The SM also predicts another fundamental boson, the Higgs boson, associated with a field that imparts mass to some of the other fundamental particles. In the SM, the Higgs boson has a spin of 0; the experimental results are consistent with that. **Composite bosons** include mesons, nuclei with an even number of neutrons and protons, and atoms containing an even number of protons, electrons, and neutrons.

Fermions: Particles with a half-integer spin quantum number (1/2, 3/2, 5/2, ...); they obey

Fermi-Dirac statistics. Multiple fermions cannot occupy the same quantum state because of the Pauli exclusion principle. They can be fundamental particles (quarks and leptons), composite particles such as the proton or neutron, nuclei containing an odd number of protons and neutrons, or atoms containing an odd number of protons, electrons, and neutrons.

Other Particles

Hadrons: Composite particles made up of quarks and/or antiquarks that are held together by gluons, the carriers of the strong force.

Mesons: Composite particles made up of a quark and an antiquark. All mesons are hadrons. Mesons are short-lived and decay quickly after they are formed.

Baryons: Composite particles made up of three quarks or three antiquarks. All baryons are hadrons. Protons and neutrons are baryons.

...

Use of natural units (GeV etc.): Einstein's relation linking energy, mass, and momentum is given by $E^2 = p^2c^2 + m^2c^4$. Hence, if energy E is measured in electron volts (eV), then momentum p is measured in eV/c and mass m in eV/c^2 . The electron volt is the amount of energy gained

by an electron when traversing a potential difference of 1 V. One GeV is 1,000,000,000 eV. In particle physics, it is common to use a system of "natural units" with c , the speed of light, set equal to 1, so that all three quantities (energy, mass, and momentum) can be expressed in eV.

Decay channel and final state: Many particles produced in proton-proton collisions at the LHC are unstable and decay almost instantaneously into other particles, which may themselves decay further. Just as a vending machine might return the same amount of change using different combinations of coins, a particle can decay into different combinations of other particles. These sets of secondary particles represent different decay channels. Detectors such as ATLAS and CMS may not observe the original particles at all, but rather the detectable collection of secondary particles from their decays that exists long enough to be detected. This detectable collection of particle species is called the final state. The Higgs particle, for example, is unstable and has many decay channels, each having a certain probability to occur called the branching ratio or branching fraction. The sum of all branching ratios is equal to 1. The branching ratios of the Higgs boson depend on the mass of the Higgs boson and are precisely predicted in the SM. For example, for a SM Higgs boson (H) of mass 125 GeV, the discovery decay channels $H \rightarrow WW$, $H \rightarrow ZZ$, and $H \rightarrow \gamma\gamma$ have branching ratios of $B(H \rightarrow WW) = 0.14$, $B(H \rightarrow ZZ) = 0.016$, and $B(H \rightarrow \gamma\gamma) = 0.0023$. Other channels studied include decays to $b\bar{b}$ and $\tau^+\tau^-$, which have branching ratios $B(H \rightarrow b\bar{b}) = 0.58$ and $B(H \rightarrow \tau^+\tau^-) = 0.064$.

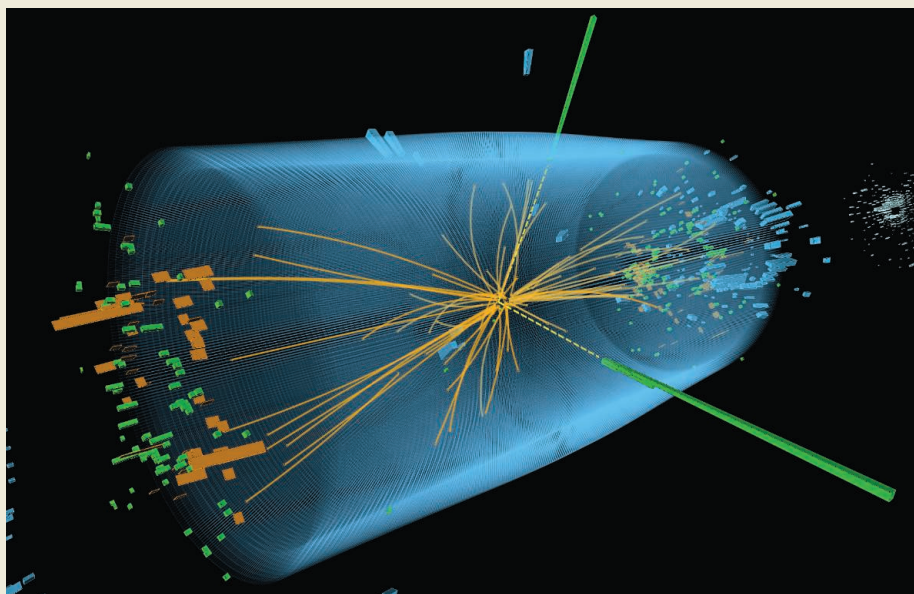
Invariant mass: Particle physicists use the word "mass" to refer to the quantity m both in Newton's second law of motion, $F = ma$, and in Einstein's equation, $E = mc^2$ (in which E must be interpreted as the energy of the particle

A reconstructed event in the CMS detector. The event shows the possible decay of the Higgs boson to a pair of photons (dashed yellow and green lines). Solid yellow lines represent the charged particles also produced in the same collision. The event could also be due to background processes.

in its rest frame and c is the speed of light in vacuum). When a particle decays and hence no longer exists, its mass before the decay can be calculated from the energies and momenta of the decay products. The inferred mass is independent of the reference frame in which the energies and momenta are measured, so that the mass is called “invariant.”

Cross section: A quantity proportional to the probability for a specified reaction (such as the creation of a new particle) to occur; for example, when the two proton beams collide as in the LHC. The name reflects the origin of the concept in classical mechanics (geometrical cross-sectional area of an object, which could be hit by a beam), but in particle physics the probabilities are determined from quantum mechanics. At the LHC, cross sections are typically expressed in nanobarns (nb), picobarns (pb), and femtobarns (fb). One barn corresponds to a cross-sectional area of 10^{-28} m^2 . For example, the cross section for the production of a SM Higgs boson of mass 125 GeV at the LHC in proton-proton collisions at the center-of-mass energy of 7 TeV is about 15 pb: $\sigma(\text{pp} \rightarrow \text{H}) = 15 \text{ pb} = 15,000 \text{ fb}$. At the center-of-mass energy of 8 TeV, the cross section is about 25% higher.

Luminosity: Instantaneous luminosity $\mathcal{L}$ gives a measure of the rate of collisions occurring

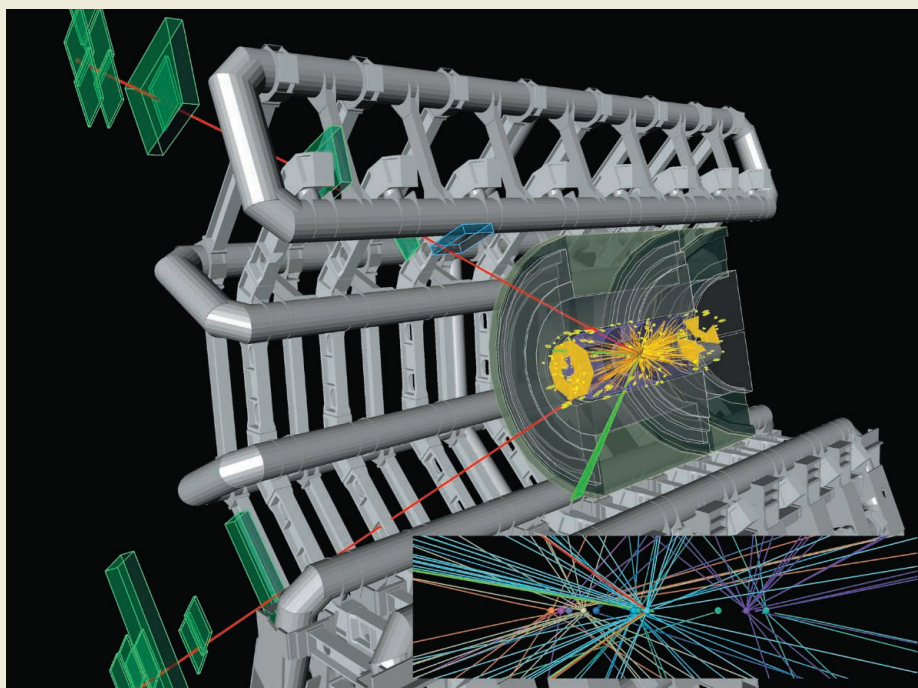


in a particle collider, based on how intense the circulating beams of particles are and how squeezed in space they are at the point of collision. Although it does not mean that all those particles will collide, squeezing more particles into a narrower space makes it more likely that they will. Instantaneous luminosity is measured in units of $\text{cm}^{-2} \text{ s}^{-1}$. Integrated luminosity is obtained by summing the product of a given time interval with the instantaneous luminosity in that interval, over time: $L = \int \mathcal{L} dt$. Integrated luminosity L is often measured in a unit called inverse femtobarn ($\text{fb}^{-1} = 10^{-39} \text{ cm}^{-2}$), which is equivalent to about 100 trillion (10^{14}) proton-proton collisions at the LHC. The number of events (N) produced in these collisions for a

given process is calculated as the product of the cross section σ for that process multiplied by the integrated luminosity L ($N = L \cdot \sigma$). For example, for a proton-proton collision run at 7 TeV resulting in an integrated luminosity of $L = 1 \text{ fb}^{-1}$, the average number of SM Higgs bosons produced in the process $\text{pp} \rightarrow \text{H}$ followed by the decay $\text{H} \rightarrow \gamma\gamma$ is $N(\text{H} \rightarrow \gamma\gamma) = L \times \sigma(\text{pp} \rightarrow \text{H}) \times B(\text{H} \rightarrow \gamma\gamma) = 1 \text{ fb}^{-1} \times 15,000 \text{ fb} \times 0.0023 = 34.5$ (a dimensionless quantity since fb^{-1} cancels with fb). In a real experiment, the number of observed events will fluctuate around the average value of 34.5, according to Poisson statistics.

Significance and the look-elsewhere effect: The probability for a background fluctuation to be at least as large as the observed maximum excess is termed the local P value, and the probability for an excess anywhere in a specified mass range is the global P value. This probability can be evaluated by generating sets of simulated data incorporating all correlations among analyses optimized for different Higgs boson masses. The global P value (for the specified region) is greater than the local P value, and this fact is often referred to as the look-elsewhere effect. Both the local and global P values can be expressed as a corresponding number of standard deviations using the one-sided Gaussian tail convention. For example, a 5σ significance tells us that the probability of the background alone fluctuating up locally by the amount observed or more is about 1 in 3 million. In particle physics, this criterion has become a convention to claim discovery but should not be interpreted literally.

A reconstructed Higgs boson decay event in the ATLAS detector. This event has two muons (red) and two electrons (green). The inset shows the reconstructed proton-proton collision vertices.



Journey in the Search for the Higgs Boson: The ATLAS and CMS Experiments at the Large Hadron Collider

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The search for the standard model Higgs boson at the Large Hadron Collider (LHC) started more than two decades ago. Much innovation was required and diverse challenges had to be overcome during the conception and construction of the LHC and its experiments. The ATLAS and CMS Collaboration experiments at the LHC have discovered a heavy boson that could complete the standard model of particle physics.

One of the remarkable achievements of 20th-century science is the revelation that a large number of natural phenomena that characterize the world around us can be described in terms of underlying principles of great simplicity and beauty. The standard model (SM) of particle physics is built upon those principles. The SM comprises quarks and leptons as the building blocks of matter, and describes their interactions through the exchange of force carriers: the photon for electromagnetic interactions, the W and Z bosons for weak interactions, and the gluons for strong interactions. Quarks are bound by the strong interaction into protons and neutrons; protons and neutrons bind together into nuclei; electrons bind to nuclei by electromagnetic interaction to form atoms, molecules, and matter. The electromagnetic and weak interactions are unified in the electroweak theory (1–3). Although integrating gravity into the model remains a fundamental challenge, the SM provides a beautiful explanation, from first principles, of much of Nature that we observe directly.

The SM has been tested by many experiments over the past four decades and has been shown to successfully describe high-energy particle interactions. The simplest and most elegant way to construct the SM would be to assert that all fundamental particles are massless. However, we know this to be untrue, otherwise atoms would not have formed and we would not exist. The question of the origin of mass of fundamental particles is the same as the one that is posed in the unified electroweak theory: Why does the photon remain strictly massless while its close cousins, the W and Z bosons, acquire a

mass some 100 times that of the proton? To generate mass of the W and Z bosons, the electroweak gauge symmetry must somehow be broken. For protons or neutrons, which are composite particles, most of the mass arises from the mass equivalent of the internal energy due to the intense binding field of the strong interactions.

Nearly 50 years ago, a mechanism was proposed for spontaneously breaking this symmetry (4–9) involving a complex scalar quantum field that permeates the entire universe. This new field has the same quantum numbers as the vacuum. The quantum of this field is called the Higgs boson. In addition to imparting mass to the W and Z bosons, this scalar field would also impart masses to the quarks and leptons, all in proportion to their coupling to this field. After the discovery of the W and Z bosons in the early 1980s, the search for the Higgs boson, considered to be an integral part of the SM, became a central theme in particle physics. The discovery of the Higgs boson would establish the existence of this field, leading to a revolutionary step in our understanding of how Nature works at the fundamental level. The elucidation of this mass-generating mechanism became one of the primary scientific goals of the Large Hadron Collider (LHC).

The mass of the Higgs boson (m_H) is not predicted by theory. Below $m_H = 600$ GeV, previous direct searches at the Large Electron Positron collider (LEP), the Tevatron, and the LHC were unable to exclude mass regions between 114 and 130 GeV (10–14). Furthermore, in December 2011, the ATLAS and CMS Collaborations reported an excess of events near a mass of 125 GeV (13, 14). The Tevatron experiments, CDF and D0, recently reported an excess of events in the range 120 to 135 GeV (15).

In July 2012, the discovery of a new heavy boson with a mass around 125 GeV was an-

nounced at CERN by the ATLAS and CMS experiments (16, 17), and the current data are consistent with the expectation for a Higgs boson. Here, we present an overview of the search for the Higgs boson and highlight some of the exciting implications. Despite its incredible success the SM is still considered incomplete. A number of questions remain: Why would the mass of the Higgs boson be only $\sim 10^2$ GeV? What is dark matter? How does the matter-antimatter asymmetry arise? How is gravity to be included? Physics beyond the SM has been much discussed over the past few decades, and such physics might manifest itself via the production of exotic particles such as superparticles from a new symmetry (supersymmetry), heavy Z-like bosons in grand unified theories or theories with extra space-time dimensions.

Design challenges. In the 1980s, it was clear that new accelerators were needed that could reach energies beyond those that had allowed the discovery of many of the subnuclear particles within the SM. Several ideas were vigorously discussed concerning the accelerators and detector concepts most capable of tackling the major open questions in particle physics, often paraphrased as the “known unknowns,” and possibly discovering new physics beyond the SM, the “unknown unknowns.” Finding the Higgs boson was clearly a priority in the first category and was expected to be challenging. The mass of the Higgs boson (m_H) is not predicted by theory and could be as high as 1000 GeV (1 TeV). This required a search over a broad range of mass, ideally suiting an exploratory machine such as a high-energy proton-proton (pp) collider. The suitability arises from the fact that the energy carried by the protons is distributed among its constituent partons (quarks and gluons), allowing the entire range of masses to be “scanned” at the same time. The main mechanisms predicted to produce the Higgs boson involve the combination of these subnuclear particles and force carriers.

The accelerator favored at CERN to probe the TeV energy scale was a pp collider. The required energy of the collider can be estimated by considering the reaction in which a Higgs boson is produced with a mass, m_H , of 1 TeV. This happens via the WW fusion production mechanism. A quark from each of the protons radiates a W boson with an energy of ~ 0.5 TeV, implying that the radiating quark should carry an energy of ~ 1 TeV so as to have a reasonable probability of emitting such a W boson. Hence, the proton should have an energy of $\sim 6 \times 1$ TeV, as the average energy carried by a quark inside the proton is about one-sixth of the proton energy. The LHC (18, 19) was designed to accelerate protons to 7 TeV, an order of magnitude higher than the most powerful available accelerator, with an instantaneous pp collision rate of 800 million per second.

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It was proposed to build the new accelerator in the existing underground tunnel of LEP. Because the radius of the tunnel is fixed, the value of the magnetic field of the dipole-bending magnets had to be ~ 8.5 T to hold in orbit the 7-TeV protons. The main challenges for the accelerator were to build ~ 1200 superconducting dipoles, each 15 m in length, able to reach this magnetic field; the large distributed cryogenic plant to cool the magnets and other accelerator structures; and control systems for the beams. The stored energy in each of the beams at nominal intensity and energy is 350 MJ, equivalent to more than 80 kg of TNT. Hence, if the beam is lost in an uncontrolled way, it can do considerable damage to the machine components, which would result in months of down time [see (18, 19) for further details].

The LHC was approved in 1994 and construction began in 1998. A parallel attempt to build an accelerator that could reach even higher energies was made with the Superconducting SuperCollider (SSC) in Texas in the late 1980s but was canceled in 1993 during the early stages of construction.

This search for the Higgs boson also provided a stringent benchmark for evaluating the physics performance of various experiment designs under consideration some 20 years ago. The predicted rate of production ($= \mathcal{L} \cdot \sigma$, where $\mathcal{L}$ is the instantaneous luminosity of the colliding beams, measured in units of $\text{cm}^{-2} \text{s}^{-1}$, and σ is the cross section of the production reaction, measured in units of cm^2) and natural width ($\Gamma = \hbar/\tau$, where τ is the lifetime, and $\hbar$ is Planck's constant divided by 2π) of the SM Higgs boson vary widely over the allowed mass range (100 to 1000 GeV). Once produced, the Higgs boson disintegrates immediately in one of several ways (decay modes) into known SM particles, depending on its mass. A search had to be envisaged not only over a large range of masses but also many possible decay modes: in pairs of photons, Z bosons, W bosons, τ leptons, and b quarks. Not only is the putative SM Higgs boson rarely produced in the proton collisions, it also rarely decays into particles that are the best identifiable signatures of its production at the LHC: photons, electrons, and muons. The rarity is illustrated by the fact that Higgs boson production and decay to one such distinguishable signature ($H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$, where $[\ell]$ is a charged lepton, either a muon or an electron) happens roughly only once in 10 trillion pp collisions. This means that a large number of pp collisions per second must be studied; the current operating number is around 600 million per second, corresponding to an instantaneous luminosity of $7 \times 10^{33} \text{ cm}^{-2} \text{s}^{-1}$. Hence, the ATLAS and CMS detectors operate in the harsh environment created by this huge rate of pp collisions.

A saying prevalent in the late 1980s and early 1990s captured the challenge that lay ahead: "We think we know how to build a high-energy, high-luminosity hadron collider—but we don't have the technology to build a detector for it." The role of the search for the Higgs boson at the LHC in influencing the design of the ATLAS and CMS experiments, and the experimental challenges associated with operating them at very high instantaneous collision rates, are described in (20).

Different values of m_H place differing demands on the detectors, none more stringent than in the mass range $m_H < 2 \times m_Z$ (where the mass of the Z boson $m_Z = 90$ GeV). In designing the LHC experiments, considerable emphasis was placed on this region. At masses below ~ 140 GeV, the SM Higgs boson is produced with a spread (natural width) of mass values of only a few MeV (i.e., a few parts in 10^5) such that the width of any observed mass peak would be entirely dominated by instrumental mass resolution. The Higgs boson decay modes generally accepted to be most promising in this region were those into two photons, occurring a few times in every thousand Higgs boson decays, and those, occurring even less often, into a pair of Z bosons, each of which in turn decays into a pair of two oppositely charged leptons (electrons or muons). However, in a detector with a very good muon momentum resolution and electron/photon energy resolution, the invariant mass of the parent Higgs bosons could be measured precisely enough with the prospect of seeing a narrow peak, over background, in the distribution of the invariant masses of the decay particles (e.g., two photons or four charged leptons). Early detailed studies can be found in (21, 22).

Other signatures are associated with a Higgs boson. Most of these signatures are plagued by larger backgrounds, as the signal characteristics are less distinctive. For example, some of these signatures include narrow sprays of particles, known as "jets," resulting from the fragmentation of quarks. These represent the most likely final states from the decay of a SM Higgs boson, but in a hadron collider they are overwhelmed by the copious production from known SM processes. Among these jets are b quark jets characterized by a short (submillimeter) flight path in the detector before disintegrating. Finally, neutrinos can be produced, which, as neutral weakly interacting leptons, leave the detector without direct trace. Energy balance in the transverse plane of the colliding protons appears to be violated, as the neutrino energy is not measured, leading to the signature of missing transverse energy (E_T^{miss}). For example, when a Higgs boson decays into two Z bosons, one can decay into a charged lepton pair and the other into a pair of neutrinos, leaving as final state an oppositely charged lepton pair and E_T^{miss} .

The conditions in hadron colliders are more ferocious than in electron-positron colliders. For example, at the LHC ~ 1000 charged particles from ~ 20 pp interactions emerge from the interaction region in every crossing of the proton bunches. Highly granular detectors (to give low probability of a given cell or channel registering a hit or energy), with fast response and good time resolution, are required. Tens of millions of detector electronic channels are hence required, and these must be synchronized to cleanly separate the different "bursts" of particles emerging from the interaction point every 25 ns. The enormous flux of particles emerging from the interaction region leads to high radiation levels in the detecting elements and the associated front-end electronics. This presented challenges for detectors and electronics not previously encountered in particle physics.

The counterrotating LHC beams are organized in 2808 bunches comprising some 10^{11} protons per bunch separated by 25 ns, leading to a bunch crossing rate of ~ 40 MHz (the LHC accelerator currently operates at 50-ns bunch spacing with 1380 bunches). The event selection process ("trigger") must reduce this rate to ~ 0.5 kHz for storage and subsequent detailed offline analysis. New collisions occur in each crossing, and a trigger decision must be made for every crossing. It is not feasible to make a trigger decision in 25 ns; it takes about 3 μs . During this time the data must be stored in pipelines integrated into onboard (front-end) electronics associated with almost every one of 100 million electronic channels in each experiment. This online event selection process proceeds in two steps: The first step reduces the rate from ~ 40 MHz to a maximum of 100 kHz and comprises hardware processors that use coarse information from the detectors; upon receipt of a positive trigger signal (set by high-momentum muons or high-energy deposits in calorimeters; see below), the data from these events are transferred to a processor farm, which uses event reconstruction algorithms operating in real time to decrease the event rate to ~ 0.5 kHz before data storage. The tens of petabytes that are generated per year per experiment are distributed to scientists located across the globe and motivated the development of the so-called Worldwide LHC Computing Grid (WLCG) (23).

Timeline and general features of the ATLAS and CMS experiments. To accomplish the physics goals, new detector technologies had to be invented and most of the existing technologies had to be pushed to their limits. Several detector concepts were proposed; two complementary ones, ATLAS and CMS, were selected in 1993 after peer review to proceed to detailed design (24–27). These designs were fully developed, and all elements prototyped and tested, over many years before construction commenced around

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1997. Today each experiment comprises more than 3000 scientists and engineers from around 180 universities and laboratories in around 40 countries. Table 1 provides a timeline of these developments.

The typical form of a collider detector is a “cylindrical onion” containing four principal layers. A particle emerging from the collision and traveling outward will first encounter the inner tracking system, immersed in a uniform magnetic field, comprising an array of pixels and microstrip detectors. These measure precisely the trajectory of the spiraling charged particles and the curvature of their paths, revealing their momenta. The stronger the magnetic field, the higher the curvature of the paths, and the more precise the measurement of each particle’s momentum. The energies of particles are measured in the next two layers of the detector, the electromagnetic (em) and hadronic calorimeters. Electrons and photons will be stopped by the em calorimeter; jets will be stopped by both the em and hadronic calorimeters. The only known particles that penetrate beyond the hadron calorimeter are muons and neutrinos. Muons, being charged particles, are tracked in dedicated muon chambers. Their momenta are also measured from the curvature of their paths in a magnetic field. Neutrinos escape detection, and their presence gives rise to E_T^{miss} .

ATLAS and CMS have differing but complementary designs (28, 29). The single most important aspect of the overall design is the choice of the magnetic field configuration for measuring the muons. The two basic configurations are solenoidal and toroidal, in which the magnetic field is parallel or azimuthal to the beam axis, respectively. The CMS has a superconducting high-field solenoid with a large ratio of length to inside diameter; ATLAS has a superconducting air-core toroid. These are the two largest magnets of their kind and hold a stored energy of up to 3 GJ. In both magnets a current of ~20 kA flows through the superconductor. The CMS solenoid additionally provides the magnetic field for the inner tracking system, whereas ATLAS has an additional solenoid magnet to carry out the same function.

At the nominal pp collision rate, the particle flux varies from $10^8 \text{ cm}^{-2} \text{ s}^{-1}$ (at a radius of $r = 4 \text{ cm}$) to $2 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ (at $r = 50 \text{ cm}$), requiring small detection cells (channels) of typical size varying from $100 \mu\text{m} \times 100 \mu\text{m}$ (pixels) to $10 \text{ cm} \times 100 \mu\text{m}$ (microstrips). The more channels there are, the easier it is to recognize the trajectories of all the charged particles produced. In practice, the number of channels is limited by the cost of the associated electronics, by the power they dissipate (which in turn requires cooling fluids), and by the need to minimize the amount of material in front of the em calorimeter. The inner tracker detectors, comprising silicon sensors and gaseous “straw” chambers, were

challenging to develop because of the need to operate in a harsh radiation environment, especially when close to the beam pipe. Radiation-hard electronics associated with each cell, with a high degree of functionality, needed to be packed into as small a space as possible, using as little material as possible.

In the early 1990s there were only two complementary possibilities for the em calorimeters that could perform in a high-radiation environment and had good enough electron and photon energy resolution to cleanly detect the two-photon decay of the SM Higgs boson at low mass: a lead–liquid argon sampling calorimeter, chosen by ATLAS, and fully sensitive dense lead tungstate scintillating crystals, chosen by CMS. Both are novel techniques, and each was tested and developed over many years before mass production could commence. The electrons and positrons in the electromagnetic showers excite atoms of lead tungstate or ionize atoms of liquid argon, respectively. The amount of light emitted, or charge collected, is proportional to the energy of the incoming electrons or photons.

The hadron calorimeters in each detector are similar and are based on known technologies: alternating layers of iron or brass absorber in which the particles interact, producing showers of secondary particles, and plastic scintillator plates that sample the energy of these showers. The total amount of scintillation light detected by the photodetectors is proportional to the incident energy.

The muon detectors used complementary technologies based on gaseous chambers: drift chambers and cathode strip chambers that provide precise position measurement (and also

provide a trigger signal in the case of CMS), and thin-gap chambers and/or resistive plate chambers that provide precise timing information as well as a fast trigger signal.

The electronics on the detectors, much of which was manufactured in radiation-hard technology, represented a substantial part of the materials cost of the LHC experiments. The requirement of radiation hardness was previously found only in military and space applications.

The construction of the various components of the detectors took place over about 10 years in universities, national laboratories, and industries, from which they were sent to CERN in Geneva. This paper can do only partial justice to the technological challenges that had to be overcome in developing, constructing, and installing all the components in the large underground caverns. All the detector elements were connected to the off-detector electronics, and data were fed to computers housed in a neighboring service cavern. Each experiment has more than 50,000 cables with a total length exceeding 3000 km, and more than 10,000 pipes and tubes for services (cooling, ventilation, power, signal transmission, etc.). Access to repair any substantial fault, or faulty connection, buried inside the experiment would require months just to open the experiments. Hence, a high degree of long-term operational reliability, which is usually associated with space-bound systems, had to be attained.

The design of the ATLAS experiment. The design of the ATLAS detector (28) was based on a superconducting air-core toroid magnet system containing ~80 km of superconductor cable in eight separate barrel coils (each $25 \text{ m} \times 5 \text{ m}$ in a “racetrack” shape) and two matching end-cap

Table 1. The timeline of the LHC project.

1984	Workshop on a Large Hadron Collider in the LEP tunnel, Lausanne.
1987	Workshop on Physics at Future Accelerators, La Thuile, Italy; the Rubbia “Long-Range Planning Committee” recommends the LHC as the right choice for CERN’s future
1990	European Committee for Future Accelerators (ECFA) LHC Workshop, Aachen, Germany (discussion of physics, technologies, and designs for LHC experiments)
1992	General Meeting on LHC Physics and Detectors, Évian-les-Bains, France (four general-purpose experiment designs presented along with their physics performance)
1993	Three letters of intent evaluated by the CERN peer review committee LHCC; ATLAS and CMS selected to proceed to a detailed technical proposal
1995	The LHC accelerator approved for construction
1996	ATLAS and CMS technical proposals approved
1997	Formal approval for ATLAS and CMS to move to construction (materials cost ceiling of 475 million Swiss francs)
1997	Construction commences [after approval of detailed engineering design of subdetectors (magnets, inner tracker, calorimeters, muon system, trigger, and data acquisition)]
2000	Assembly of experiments commences; LEP accelerator is closed down to make way for the LHC
2008	LHC experiments ready for pp collisions; LHC starts operation; an incident stops LHC operation
2009	LHC restarts operation; pp collisions recorded by LHC detectors
2010	LHC collides protons at high energy (center-of-mass energy of 7 TeV)
2012	LHC operates at 8 TeV; discovery of a Higgs-like boson

toroid systems. A field of ~ 0.5 T is generated over a large volume. The toroids are complemented with a smaller solenoid (diameter 2.5 m, length 6 m) at the center, which provides a magnetic field of 2 T.

The detector includes an em calorimeter complemented by a full-coverage hadronic calorimeter for jet and E_T^{miss} measurements. The em calorimeter is a cryogenic lead-liquid argon sampling calorimeter in a novel “accordion” geometry allowing fine granularity, both laterally and in depth, and full coverage without any uninstrumented regions. A plastic scintillator-iron sampling hadronic calorimeter, also with a novel geometry, is used in the barrel part of the experiment. Liquid argon hadronic calorimeters are used in the end-cap regions near the beam axis. The em and hadronic calorimeters have 200,000 and 10,000 cells, respectively, and are in an almost field-free region between the toroids and the solenoid. They provide fine lateral and longitudinal segmentation.

The momentum of the muons can be precisely measured as they travel unperturbed by material for more than ~ 5 m in the air-core toroid field. About 1200 large muon chambers

of various shapes, with a total area of 5000 m², measure the impact position with an accuracy better than 0.1 mm. Another set of about 4200 fast chambers are used to provide the “trigger.” The chambers were built in about 20 collaborating institutes on three continents. (This was also the case for other components of the experiment.)

The reconstruction of all charged particles, including that of displaced vertices, is achieved in the inner detector, which combines highly granular pixel ($50\text{ }\mu\text{m} \times 400\text{ }\mu\text{m}$ elements leading to 80 million channels) and microstrip ($13\text{ cm} \times 80\text{ }\mu\text{m}$ elements leading to 6 million channels) silicon semiconductor sensors placed close to the beam axis, and a “straw tube” gaseous detector (350,000 channels), which provides about 30 to 40 signal hits per track. The latter also helps in the identification of electrons using information from the effects of transition radiation.

The air-core magnet system allows a relatively lightweight overall structure leading to a detector weighing 7000 tonnes. The muon spectrometer defines the overall dimensions of the ATLAS detector: a diameter of 22 m and a length of 46 m. Given its size and structure, the

ATLAS detector had to be assembled directly in the underground cavern. Figure 1 shows one end of the cylindrical barrel detector after about 4 years of installation work, 1.5 years before completion. The ends of four of the barrel toroid coils are visible, illustrating the eightfold symmetry of the structure.

The design of the CMS experiment. The design of the CMS detector (29) was based on a superconducting high-field solenoid, which first reached the design field of 4 T in 2006. The CMS design was first optimized to detect muons from the $H \rightarrow ZZ \rightarrow 4\mu$ decay. To identify these muons and measure their momenta, the interaction region of the CMS detector is surrounded with enough absorber material, equivalent to about 2 m of iron, to stop all the particles produced except muons and neutrinos. The muons have spiral trajectories in the magnetic field, which are reconstructed in the surrounding drift chambers. The CMS solenoid was designed to have the maximum magnetic field considered feasible at the time, 4 T. This is produced by a current of 20 kA flowing through a reinforced Nb-Ti superconducting coil built in four layers. Economic and transportation

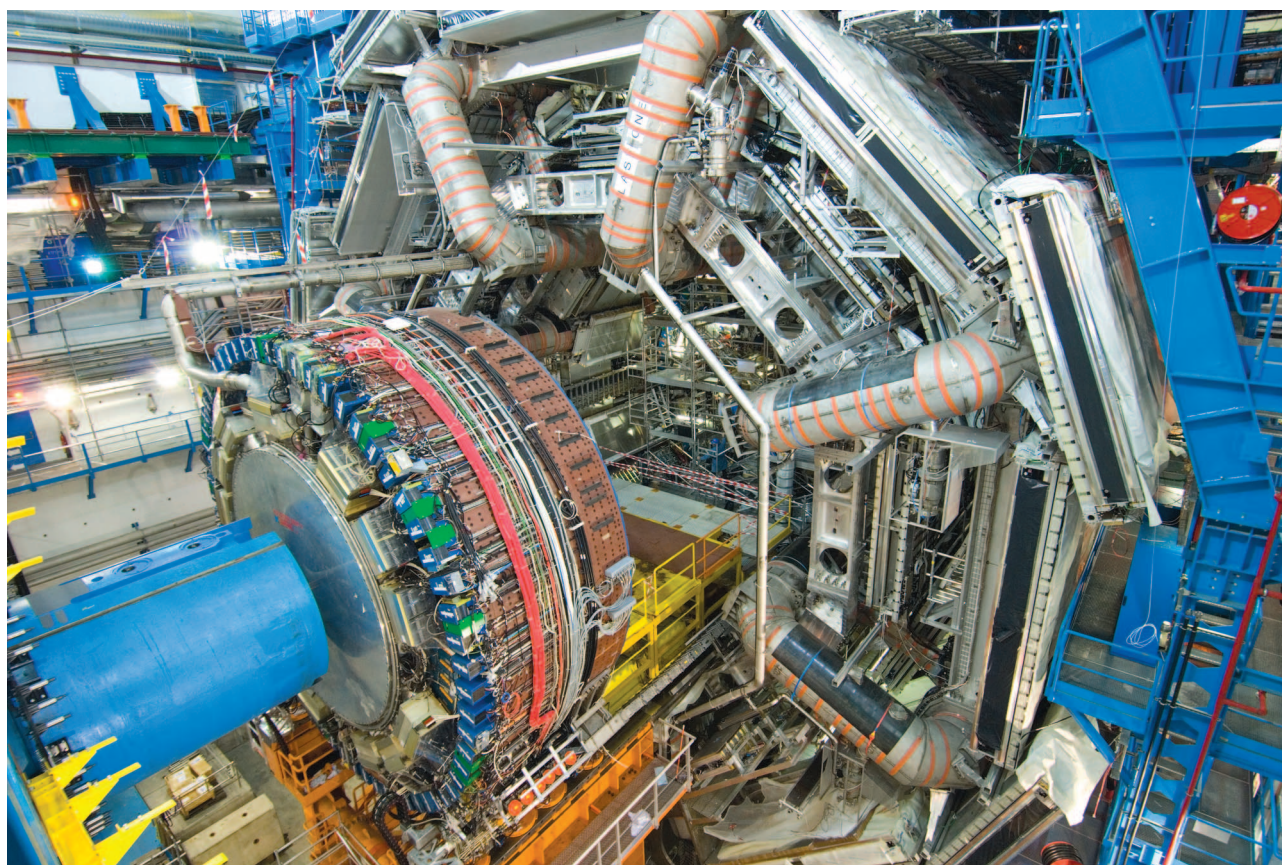


Fig. 1. Photograph of one end of the ATLAS detector barrel with the calorimeter end cap still retracted before its insertion into the barrel toroid magnet structure (February 2007 during the installation phase).

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constraints limited the outer radius of the coil to 3 m and its length to 13 m. The field is returned through an iron yoke, 1.5 m thick, which houses four muon stations to ensure robustness of measurement and full geometric coverage. The iron yoke is sectioned into five barrel wheels and three end-cap disks at each end, for a total weight of 12,500 tonnes. The sectioning enabled the detector to be assembled and tested in a large surface hall while the underground cavern was being prepared. The sections, weighing 350 to 2000 tonnes, were then lowered sequentially between October 2006 and January 2008, using a dedicated gantry system equipped with strand jacks; this represented the first use of this technology to simplify the underground assembly of large experiments.

The next design priority was driven by the search for the decay of the SM Higgs boson into two photons. This called for an em calorimeter with the best possible energy resolution. A new type of crystal was selected: lead tungstate (PbWO_4) scintillating crystal. Five years of research and development (1993–1998) were necessary to improve the transparency and the radiation hardness of these crystals, and it

took more than 10 years (1998–2008) of round-the-clock production to manufacture the 75,848 crystals—more crystals than were used in all previous particle physics experiments put together. The last of the crystals was delivered in March 2008.

The solution to charged particle tracking was to opt for a small number of precise position measurements of each charged track (~13 each with a position resolution of ~15 μm per measurement), leading to a large number of cells distributed inside a cylindrical volume 5.8 m in length and 2.5 m in diameter: 66 million silicon pixels, each $100\text{ }\mu\text{m} \times 150\text{ }\mu\text{m}$, and 9.3 million silicon microstrips ranging from ~10 $\text{cm} \times 80\text{ }\mu\text{m}$ to ~20 $\text{cm} \times 180\text{ }\mu\text{m}$. With 198 m^2 of active silicon area, the CMS tracker is by far the largest silicon tracker ever built.

Finally, the hadron calorimeter, comprising ~3000 small solid angle projective towers covering almost the full solid angle, is built from alternate plates of ~5 cm brass absorber and ~4-mm-thick scintillator plates that sample the energy. The scintillation light is detected by photodetectors (hybrid photodiodes) that can operate in the strong magnetic field. Figure 2 shows the trans-

verse view of the barrel part of CMS in late 2007 during the installation phase in the underground cavern.

Preparation of the experiments. All detector components were tested at their production sites, after delivery to CERN, and again after their installation in the underground caverns. The experiments made use of the constant flow of cosmic rays impinging on Earth. Even at depths of 100 m, there is still a small flux of muons—a few hundred per second traversing each of the experiments. The muons were used to check the whole chain from the hardware to the analysis programs of the experiments, and also to align the detector elements and calibrate their response prior to pp collisions (30, 31).

The ATLAS and CMS experiments would generate huge amounts of data (tens of petabytes of data per year; 1 PB = 10^6 GB), requiring a fully distributed computing model. The LHC Computing Grid allows any user anywhere access to any data recorded or calculated during the lifetime of the experiments. The computing system consists of a hierarchical architecture of tiered centers, with one large Tier-0 center at CERN, about 10 large Tier-1 centers at national

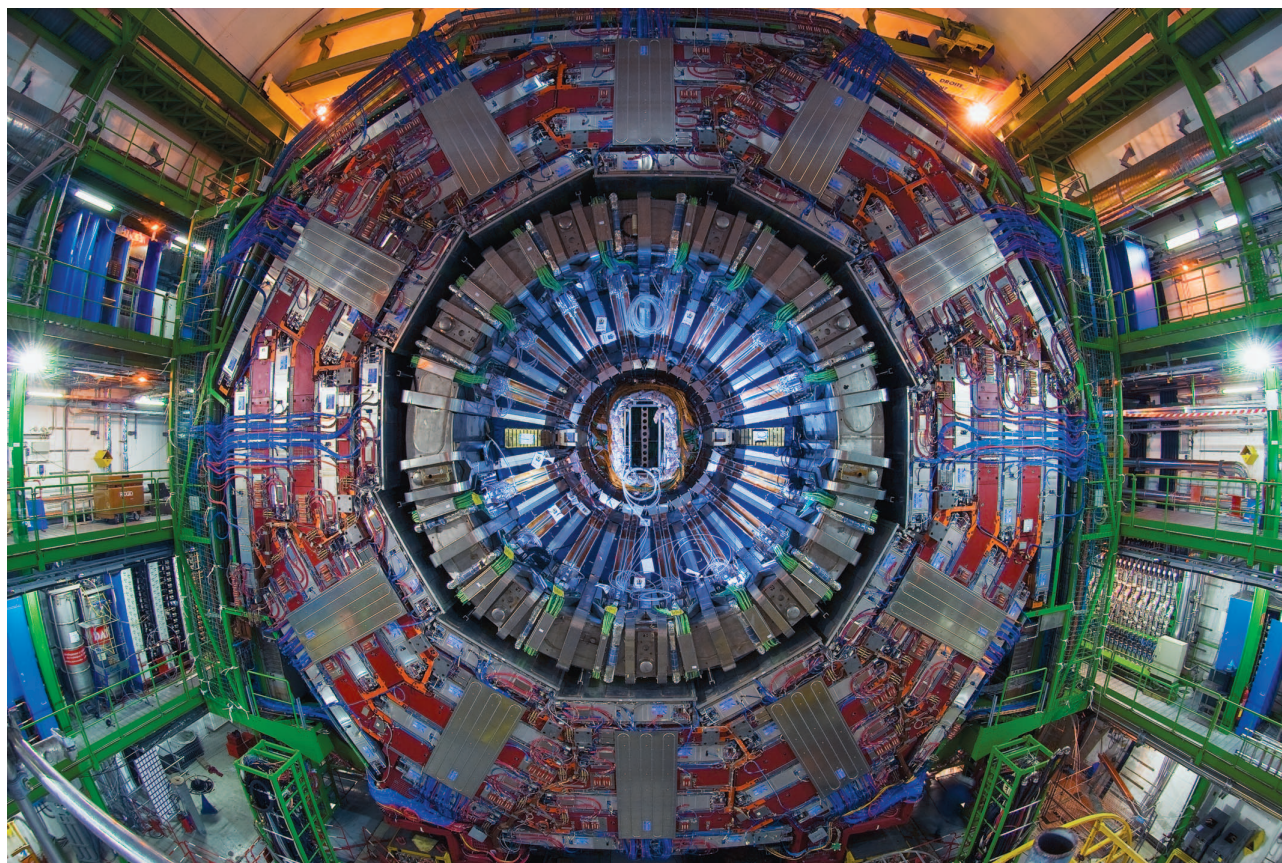


Fig. 2. Transverse section of the barrel part of CMS illustrating the successive layers of detection starting from the center where the collisions occur: the inner tracker, the crystal calorimeter, the hadron calorimeter, the superconducting coil, and the iron yoke instrumented with the four muon stations. The last muon station is at a radius of 7.4 m.

computing facilities, and about 100 Tier-2 centers at various institutes. The center at CERN receives the raw data, carries out prompt reconstruction almost in real time, and exports the raw and reconstructed data to the Tier-1 centers and also to Tier-2 centers for physics analysis. The Tier-0 centers must keep pace with the event rate of 0.5 kHz (~ 1 MB of raw data per event) from each experiment. The large Tier-1 centers provide long-term storage of raw data and reconstructed data outside of CERN (a second copy). They carry out second-pass reconstruction, when better calibration constants are available. The large number of events simulated by Monte Carlo methods and necessary for quantifying the expectations are produced mainly in Tier-2 centers.

The operation of the LHC accelerator and the experiments. The LHC accelerator began to operate on 10 September 2008. On 19 September 2008, during the final powering tests of the main dipole circuit in the last sector (3-4) to be powered up, an electrical fault in one of the tens of thousands of connections generated a powerful spark that punctured the vessel of a magnet, resulting in a large release of helium from the magnet cold mass and leading to mechanical damage to ~ 50 magnets. These were repaired in 2009, and it was decided to run the accelerator at an energy of 3.5 TeV per beam (i.e., at half the nominal energy).

The first pp collisions (at an energy of 450 GeV per beam) occurred on 23 November 2009; the first high-energy collisions (at 3.5 TeV per beam) were recorded on 30 March 2010, and since then

the collider has operated smoothly, providing the two general-purpose experiments, ATLAS and CMS, with data samples corresponding to an integrated luminosity of close to 5 fb^{-1} (fb, femtobarn) during 2011, and another 5 fb^{-1} , at the slightly higher energy of 4 TeV per beam, up to June 2012. In total, these data ($\sim 10^{15}$ pp collisions) correspond to the examination of some 10^{15} pp collisions. Typically, there are 20 overlapping pp interactions ("pile-up") in the same crossing of proton bunches as the interaction of interest. ATLAS and CMS have recorded $\sim 95\%$ of the collision data delivered with the LHC operating in stable conditions. In all, 98% of the roughly 100 million electronic readout channels in each experiment have been performing at design specification. This outstanding achievement is the result of a constant and dedicated effort by the teams of physicists, engineers, and technicians responsible for the hardware, software, and maintenance of the detectors. This efficient operation of the accelerator and the experiments has led to the discovery of the Higgs-like boson soon after the first pp collisions at high energy.

The ATLAS and CMS experiments started recording high-energy pp collisions in March 2010 after a preliminary low-energy run in the autumn of 2009. Many SM processes, including inclusive production of quarks (seen as hadronic jets), bottom quarks, top quarks, and W and Z bosons, have been measured with high precision. These measurements, in a previously unexplored energy region, confirm the predictions of the SM. It is essential to establish this agreement before any claims for new physics can

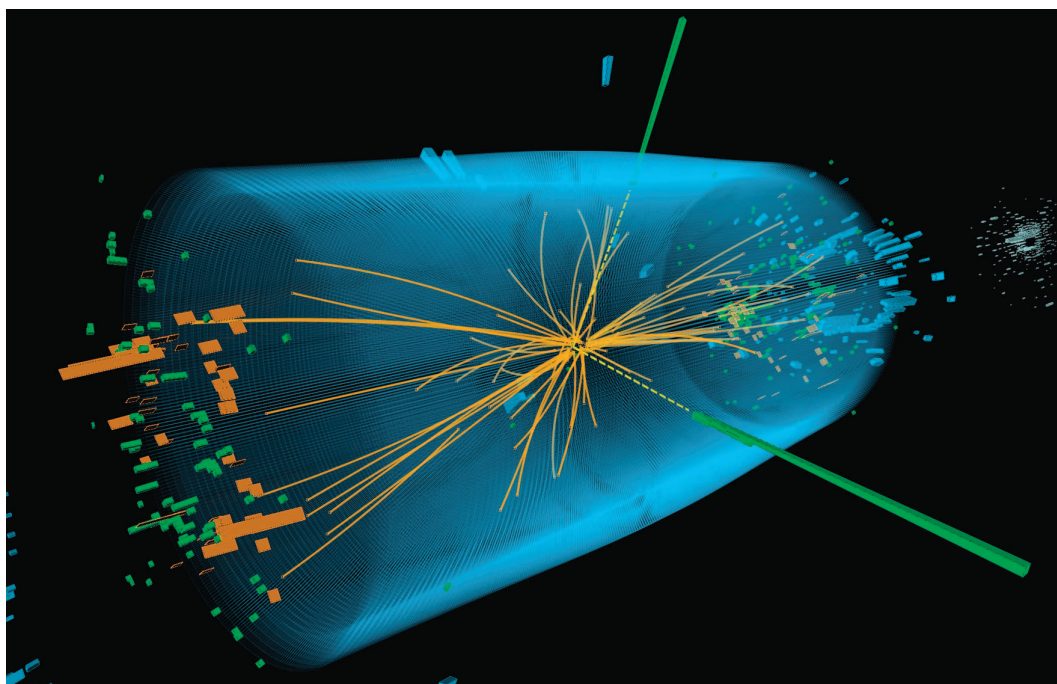
be made, as SM processes constitute large backgrounds to new physics.

Extensive searches for new physics beyond the SM have also been performed. New limits have been set on quark substructure, supersymmetric particles (e.g., disfavoring at 95% CL gluino masses below 1 TeV in simple models of supersymmetry), potential new bosons (e.g., disfavoring at 95% CL new heavy W-like W' and Z-like Z' bosons with masses below 2 TeV for couplings similar to the ones for the known W and Z bosons), and even signs of TeV-scale gravity (e.g., disfavoring at 95% CL black holes with masses below 4 TeV).

Undoubtedly, the most striking result to emerge from the ATLAS and CMS experiments is the discovery of a new heavy boson with a mass of ~ 125 GeV. The analysis was carried out in the context of the search for the SM Higgs boson.

For m_H around 125 GeV, and from the number of collisions examined, some 200,000 Higgs bosons would have been produced in each experiment. Folding in the branching fraction, each experiment expected to identify a comparatively tiny number of signal events (e.g., a few hundred two-photon events or tens of four-lepton events) from a hypothetical Higgs boson, before including factors of efficiency. The four-charged-lepton mode ($H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$) offers the promise of the purest signal ($S/B \sim 1$, where S is the number of expected signal events and B is the number of expected background events) and has therefore been called the "golden channel."

Fig. 3. Event recorded with the CMS detector on 13 May 2012 at a proton-proton center-of-mass energy of 8 TeV. The event shows characteristics expected from the decay of the SM Higgs boson to a pair of photons (dashed yellow lines and green towers). Solid yellow lines represent the reconstructed trajectories of the charged particles produced in addition to the two photons in the same collision. The event could also be due to known SM background processes.



The Higgs Boson

The search for the Higgs boson is carried out in a variety of modes. Below, we give some details of the two modes that have the best invariant mass resolution and had played a particularly important role in the design of the ATLAS and CMS experiments.

$H \rightarrow \gamma\gamma$. In our detectors, the signature of the $H \rightarrow \gamma\gamma$ decay mode is a pair of isolated photons each with a high transverse momentum of ~ 30 GeV or higher. Transverse momentum is the component of the momentum vector projected onto the plane perpendicular to the beams. Figure 3 shows such an event recorded with the CMS detector.

Events containing two isolated photon candidates were selected with the goal of identifying a narrow peak in the diphoton invariant mass distribution superimposed on a large background. This background arises from two sources: the dominant and irreducible one from a variety of SM processes, and a reducible background where one or both of the reconstructed photon candidates originate from misidentification of jet fragments.

The criteria to distinguish real photons from those coming from jet fragmentation (labeled “fake photons”) depend on the detector technol-

ogies of the two experiments. Both experiments are able to reject fake photons such that their contribution to the background is only 25% of the total. The size of this contribution was the subject of much debate in the 1990s, and the low value has been attained through the design of the em calorimeters and the rejection power of the associated analyses.

To enhance the sensitivity of the analysis, candidate two-photon events were separated into many mutually exclusive categories of different expected S/B ratios. These categories are defined on the basis of the expected properties

Fig. 4. (A) The two-photon invariant mass distribution in ATLAS of selected candidate events weighted by the S/B value of the category in which it falls. The 7-TeV and 8-TeV data are combined and correspond to a total integrated luminosity of 10.7 fb^{-1} . (B) The two-photon invariant mass distribution in CMS of selected candidate events weighted by the $S/(S+B)$ value of the category in which it falls. The 7-TeV and 8-TeV data are combined and correspond to a total integrated luminosity of 10.4 fb^{-1} .

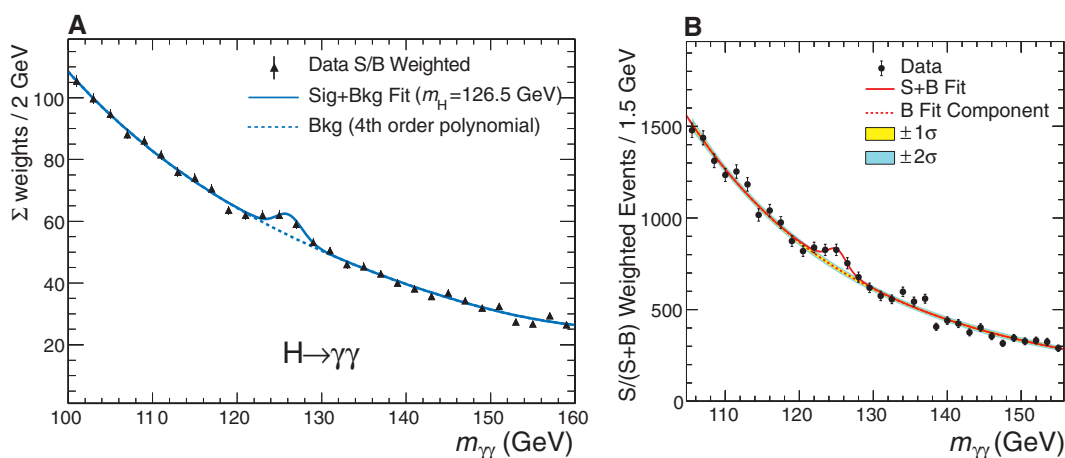
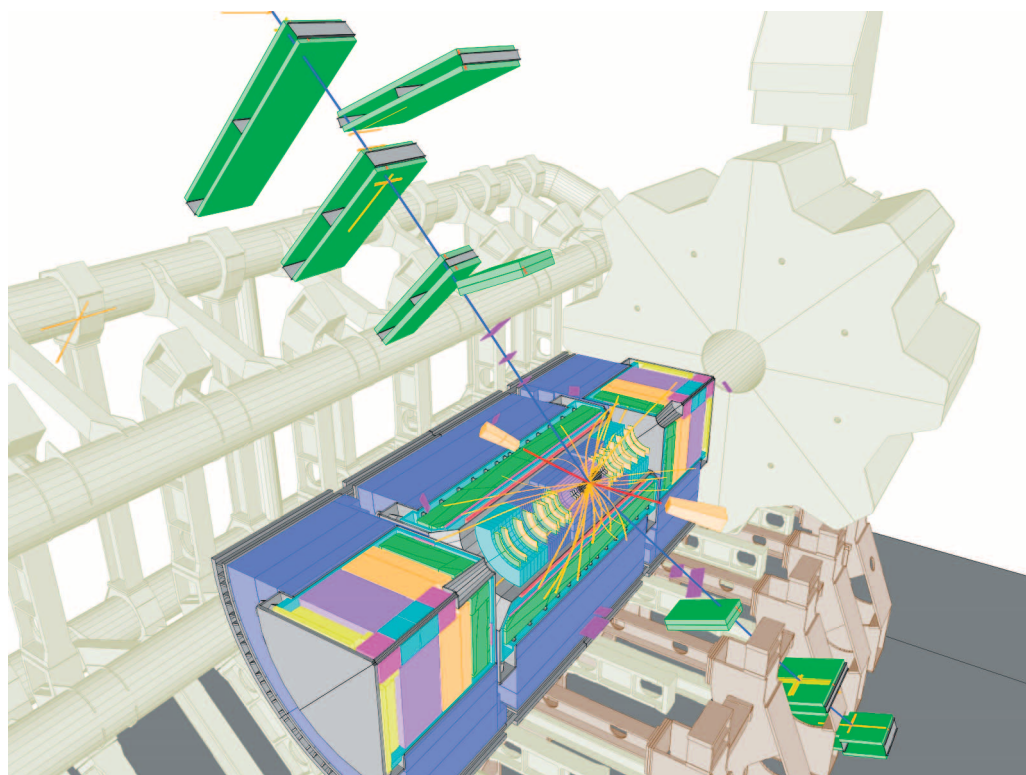


Fig. 5. Event recorded with the ATLAS detector on 30 May 2011 at a proton-proton center-of-mass energy of 7 TeV. The event shows characteristics expected from the decay of the SM Higgs boson to a pair of Z bosons, which in turn decay to a pair of electrons (red tracks and yellow towers) and a pair of muons (blue tracks and muon chamber hits in green). The invariant mass of the $2e2\mu$ system is 124.3 GeV. The event could also be due to known SM background processes.



of the reconstructed photons and on the presence of two jets expected to accompany a Higgs boson produced through the vector-boson fusion process, a particularly sensitive category. The analysis of events in each category represented a separate measurement, with a specific mass resolution and background, and the results from each category were statistically combined through a procedure that used likelihood analysis.

The distributions of the two-photon invariant masses, weighted by category, are shown in Fig. 4 for ATLAS and CMS, respectively, along with the best fit of a signal peak on top of a continuum background. The weight chosen was proportional to the expected S/B in the respective category.

An excess of events, over the background, was observed at a mass of ~ 125 GeV by both experiments, corresponding to a local significance of 4.5 standard deviations (σ) for ATLAS and 4.1 σ for CMS.

$H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$. The signature of the $H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$ decay mode is two pairs of oppositely

charged isolated leptons (electrons or muons). The main background arises from a small continuum of known and nonresonant production of Z boson pairs. Figure 5 shows an event recorded with the ATLAS detector with the characteristics expected from the decay of the SM Higgs boson to a pair of Z bosons, one of which subsequently decays into a pair of electrons and the other into a pair of muons.

For a Higgs boson with a mass below twice the mass of the Z boson, one of the lepton pairs will typically have an invariant mass compatible with the Z boson mass (~ 91 GeV), whereas the other one will have a considerably lower mass, called “off-mass shell.” Because there are differences in the instrumental backgrounds and in the mass resolutions for the three possible combinations of electron and muon pairs ($4e$, 4μ , and $2e2\mu$), the searches were made in these independent subchannels and then combined statistically with a likelihood procedure. In the case of CMS, the angular distribution of the four leptons is included in the likelihood.

Figure 6 shows the four-lepton invariant mass distribution for the ATLAS and CMS experiments, in each case for the combination of all the channels ($4e$, 4μ , and $2e2\mu$). The peak near 90 GeV corresponds to the expected but rare decay of Z bosons to four leptons ($Z \rightarrow \ell\ell\ell\ell$). The rate is higher in the CMS experiment than in ATLAS because of differing kinematic criteria applied to the four leptons. Both experiments observe a small but significant excess of events around an invariant mass of about 125 GeV above the expected continuum background, with a spread as expected from the mass resolution and statistical fluctuations corresponding to a local significance of 3.4 σ and 3.2 σ in ATLAS and CMS, respectively.

Combined results. The ATLAS and CMS experiments have both studied more Higgs boson decay modes than described in this paper, as discussed in the associated papers in this issue and (16, 17). Figure 7 shows the combined statistical significance observed for the different Higgs mass hypotheses by the ATLAS and CMS experiments, respectively. The largest

Fig. 6. (A and B) The distribution of the four-lepton invariant mass, $m_{\ell\ell\ell\ell}$, in ATLAS (A) and CMS (B) for the selected candidates relative to the background expectation. The signal expectation for a SM Higgs boson with $m_H = 125$ GeV is also shown. The 7-TeV and 8-TeV data are combined and correspond to a total integrated luminosity of 10.7 fb $^{-1}$ for ATLAS and 10.4 fb $^{-1}$ for CMS.

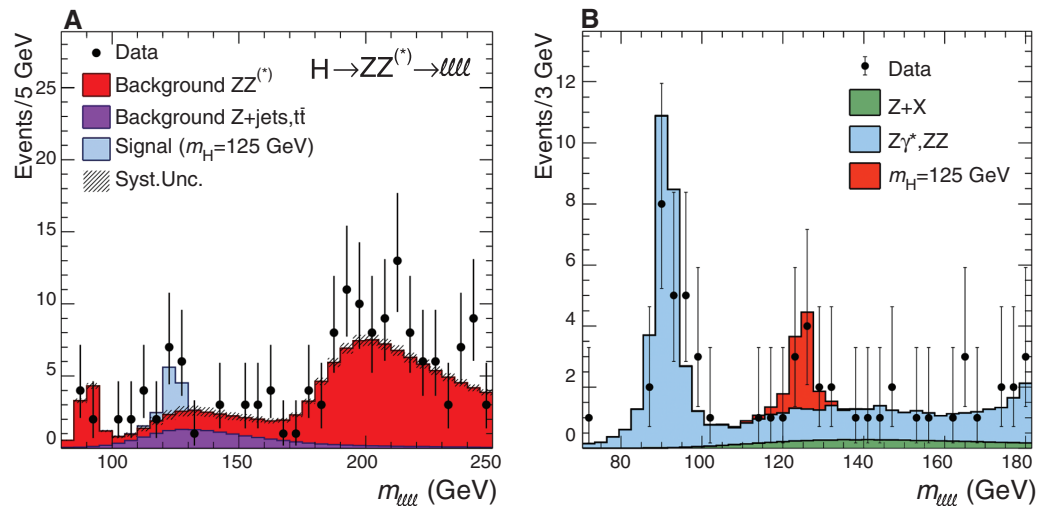
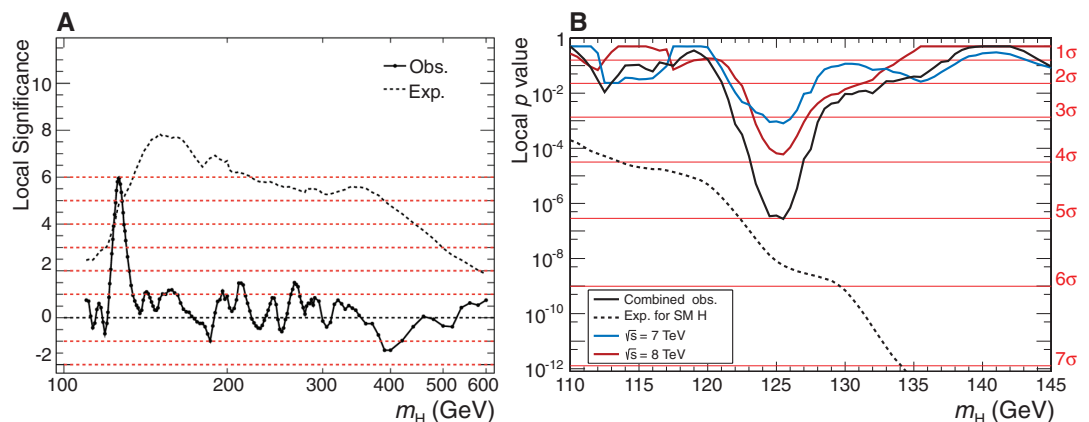


Fig. 7. (A and B) Combined search results in ATLAS (A) and CMS (B) detectors as a function of m_H for the observed and expected local significance based on the estimated background from SM processes. The 7-TeV and 8-TeV data are combined and correspond to a total integrated luminosity of 10.4 to 10.7 fb $^{-1}$ for ATLAS and 10.4 fb $^{-1}$ for CMS.



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local significance is observed for a SM Higgs boson mass of $m_H = 126.5$ and 125.5 GeV, where it reaches 6.0σ and 5.0σ , corresponding to a background fluctuation probability of 2×10^{-9} and 5×10^{-7} for the ATLAS and CMS experiments, respectively. The expected local significance in the presence of a SM Higgs boson signal at these masses is found to be 4.9σ for the ATLAS experiment and 5.8σ for the CMS experiment. The evidence for a new particle is strengthened by the observation in two different experiments, comprising complementary detectors, operating independently.

In both experiments the excess was most significant in the two decay modes $\gamma\gamma$ and ZZ . These two decay modes indicate that the new particle is a boson; the two-photon decay implies that its spin (J) is different from 1 (32, 33). Because the Higgs field is scalar, the spin of the SM Higgs boson is predicted to be zero.

Furthermore, the number of observed events is roughly equal to the number of events expected from the production of a SM Higgs boson for all the decay modes analyzed, within the errors, in both experiments. The measured value of the observed/expected ratio, for the combined data from all the decay modes, was found to be 1.4 ± 0.3 and 0.87 ± 0.23 for the ATLAS and CMS experiments, respectively. The best estimates of the masses measured by the ATLAS and CMS experiments are also consistent: 126.0 ± 0.6 GeV and 125.3 ± 0.6 GeV, respectively.

Outlook. The results from the two experiments are consistent, within uncertainties, with the expectations for the SM Higgs boson, a fundamental spin-0 (scalar) boson. Much more data need to be collected to enable rigorous testing of the compatibility of the new boson with the SM and to establish whether the properties of the new particle imply the existence of physics beyond the SM. For this boson, at a mass of ~ 125 GeV, almost all the decay modes are detectable, and hence comprehensive tests can be made. Among the remaining questions are whether the bosons have spin $J = 0$ or $J = 2$, whether their parity is positive or negative, whether they are elementary or composite, and whether they couple to particles in the exact proportion predicted by the SM [i.e., for fermions (f) proportional to m_f^2 and for bosons (V) proportional to m_V^4]. These properties are studied via the new boson's rate of decay into different final states, the angular distributions of the decay particles, and its rate of production in association with other particles such as W and Z bosons. The SM Higgs boson is predicted to be an elementary particle with $J^P = 0^+$. Much progress is expected, as by the end of 2012 the ATLAS and CMS detectors should be able to triple the amount of data used for the results presented here. The LHC will then be shut down in 2013 and 2014 to refurbish

parts of the accelerator so that it will be able to reach its full design energy (14 TeV) and enable precise measurements of the properties of the new bosons and the full exploration of the physics of the TeV energy scale, especially the search for physics beyond the SM.

It is known that quantum corrections make the mass of a fundamental scalar particle float up to the next highest physical mass scale currently known, which, in the absence of extensions to the SM, is as high as 10^{15} GeV. A favored conjecture states that this is avoided by a set of heavy particles not yet discovered. For each known SM particle there would be a partner with spin differing by half a unit; fermions would have boson partners and vice versa, in a symmetry called supersymmetry. This happens because in quantum mechanics, corrections involving fermions and bosons have opposite signs for their amplitudes and hence cancel each other. In the minimal supersymmetry model, five types of Higgs bosons are predicted to exist. Furthermore, the lightest stable neutral particle of this new family of supersymmetric particles could be the particle constituting dark matter. If, as conjectured, such particles are light enough, they ought to reveal themselves at the LHC.

The discovery of the new boson suggests that we could well have discovered a fundamental scalar field that pervades our universe. Astronomical and astrophysical measurements point to the following composition of energy-matter in the universe: $\sim 4\%$ normal matter that “shines,” $\sim 23\%$ dark matter, and the rest forming “dark energy.” Dark matter is weakly and gravitationally interacting matter with no electromagnetic or strong interactions. These are the properties carried by the lightest supersymmetric particle. Hence the question: Is dark matter supersymmetric in nature? Fundamental scalar fields could well have played a critical role in the conjectured inflation of our universe immediately after the Big Bang and in the recently observed accelerating expansion of the universe that, among other measurements, signals the presence of dark energy in our universe.

The discovery of the new boson is widely expected to be a portal to physics beyond the SM. Physicists at the LHC are eagerly looking forward to establishing the true nature of the new boson and to the higher-energy running of the LHC, to find clues or answers to some of the other fundamental open questions in particle physics and cosmology. Such a program of work at the LHC is likely to take several decades.

References and Notes

1. S. L. Glashow, *Nucl. Phys.* **22**, 579 (1961).
2. S. Weinberg, *Phys. Rev. Lett.* **19**, 1264 (1967).
3. A. Salam, in *Elementary Particle Physics: Relativistic Groups and Analyticity, Proceedings of the Eighth Nobel*

Symposium, N. Svartholm, Ed. (Almqvist & Wiskell, Stockholm, 1968), p. 367.

4. F. Englert, R. Brout, *Phys. Rev. Lett.* **13**, 321 (1964).
5. P. W. Higgs, *Phys. Lett.* **12**, 132 (1964).
6. P. W. Higgs, *Phys. Rev. Lett.* **13**, 508 (1964).
7. G. S. Guralnik, C. R. Hagen, T. W. B. Kibble, *Phys. Rev. Lett.* **13**, 585 (1964).
8. P. W. Higgs, *Phys. Rev.* **145**, 1156 (1966).
9. T. W. B. Kibble, *Phys. Rev.* **155**, 1554 (1967).
10. ALEPH, DELPHI, L3, OPAL Collaborations, and LEP Working Group for Higgs Boson Searches, *Phys. Lett. B* **565**, 61 (2003).
11. CDF and D0 Collaborations, *Phys. Rev. Lett.* **104**, 061802 (2010).
12. CDF Collaboration, D0 Collaboration, and Tevatron New Physics, Higgs Working Group, <http://arxiv.org/abs/1207.0449> (2012).
13. ATLAS Collaboration, *Phys. Rev. D* **86**, 032003 (2012).
14. CMS Collaboration, *Phys. Lett. B* **710**, 26 (2012).
15. CDF Collaboration and D0 Collaboration, *Phys. Rev. Lett.* **109**, 071804 (2012).
16. ATLAS Collaboration, *Phys. Lett. B* **716**, 1 (2012).
17. CMS Collaboration, *Phys. Lett. B* **716**, 30 (2012).
18. L. Evans, Ed., *The Large Hadron Collider, a Marvel of Technology* (EPFL Press, Lausanne, Switzerland, 2009).
19. L. Evans, P. Bryant, *JINST* **3**, S08001 (2008).
20. N. Ellis, T. S. Virdee, *Annu. Rev. Nucl. Part. Sci.* **44**, 609 (1994).
21. C. J. Seez et al., in *Proceedings of the Large Hadron Collider Workshop*, G. Jarlskog, D. Rein, Eds. Aachen, Germany, 1990, p. 474, CERN 90-10-V-2/ECFA 90-133-V-2, <http://cdsweb.cern.ch/record/215298>.
22. M. Della Negra et al., in *Proceedings of the Large Hadron Collider Workshop*, G. Jarlskog, D. Rein, Eds. Aachen, Germany, 1990, p. 509, CERN 90-10-V-2/ECFA 90-133-V-2, <http://cdsweb.cern.ch/record/215298>.
23. LHC Computing Grid, Technical Design Report CERN-LHCC-2005-024 (2005).
24. ATLAS Collaboration, ATLAS: Letter of Intent for a General-Purpose pp Experiment at the Large Hadron Collider at CERN, CERN-LHCC-92-004 (1992).
25. ATLAS Collaboration, Technical Proposal CERN-LHCC-1994-043 (1994).
26. CMS Collaboration, Letter of Intent by the CMS Collaboration for a General Purpose Detector at the LHC, CERN-LHCC-92-003 (1992).
27. CMS Collaboration, Technical Proposal CERN-LHCC-1994-038 (1994).
28. ATLAS Collaboration, *JINST* **3**, S08003 (2008).
29. CMS Collaboration, *JINST* **3**, S08004 (2008).
30. ATLAS Collaboration, *Eur. Phys. J. C* **71**, 1593 (2011).
31. CMS Collaboration, *JINST* **5**, T03001 (2010).
32. L. D. Landau, *Dokl. Akad. Nauk* **60**, 207 (1948).
33. C. N. Yang, *Phys. Rev.* **77**, 242 (1950).

Acknowledgments: The construction, and now the operation and exploitation, of the large and complex ATLAS and CMS experiments have required the talents, the resources, and the dedication of thousands of scientists, engineers, and technicians worldwide. Many have already spent a substantial fraction of their working lives on these experiments. This paper is dedicated to all our colleagues who have worked on these experiments. None of these results could have been obtained without the wise planning, superb construction, and efficient operation of the LHC accelerator and the WLCG computing.

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ARTICLE

A New Boson with a Mass of 125 GeV Observed with the CMS Experiment at the Large Hadron Collider

The CMS Collaboration*†

The Higgs boson was postulated nearly five decades ago within the framework of the standard model of particle physics and has been the subject of numerous searches at accelerators around the world. Its discovery would verify the existence of a complex scalar field thought to give mass to three of the carriers of the electroweak force—the W^+ , W^- , and Z^0 bosons—as well as to the fundamental quarks and leptons. The CMS Collaboration has observed, with a statistical significance of five standard deviations, a new particle produced in proton-proton collisions at the Large Hadron Collider at CERN. The evidence is strongest in the diphoton and four-lepton (electrons and/or muons) final states, which provide the best mass resolution in the CMS detector. The probability of the observed signal being due to a random fluctuation of the background is about 1 in 3×10^6 . The new particle is a boson with spin not equal to 1 and has a mass of about 1.25 giga-electron volts. Although its measured properties are, within the uncertainties of the present data, consistent with those expected of the Higgs boson, more data are needed to elucidate the precise nature of the new particle.

The standard model (SM) of particle physics (1–3) describes the fundamental particles, quarks and leptons, and the forces that govern their interactions. Within the SM, the photon is massless, whereas the masses of the other carriers of the electroweak force, the $W^\pm$ and Z^0 gauge bosons, are generated through a symmetry-breaking mechanism proposed by three groups of physicists (Englert and Brout; Higgs; and Guralnik, Hagen, and Kibble) (4–9). This mechanism introduces a complex scalar field, leading to the prediction of a scalar particle: the SM Higgs boson. In contrast, all known elementary bosons are vector particles with spin 1. In the SM, the scalar field also gives mass to the fundamental fermions through a Yukawa interaction (1–3). The Higgs boson is predicted to decay almost instantly to lighter particles.

The theory does not predict a specific mass for the Higgs boson. Moreover, the properties of the Higgs boson depend strongly on its mass. General arguments indicate that its mass should be less than about 1 TeV (10–13), although searches for the SM Higgs boson conducted before those at the Large Hadron Collider (LHC) have excluded the mass region below 114.4 GeV (14). Searches at the Tevatron have excluded a narrow mass region near 160 GeV (15) and recently reported an excess of events in the range from 120 to 135 GeV (16–18).

The LHC is installed in a circular tunnel 27 km in circumference and 100 m underground, strad-

dling the border between France and Switzerland, near Geneva (19). The LHC accelerates clockwise and counterclockwise beams of protons before colliding them head on. These collisions were at a total center-of-mass energy of 7 TeV in 2011 and 8 TeV in 2012, the highest energies reached to date in a particle accelerator. These high-energy collisions enable the production of new, and sometimes very heavy, particles by converting energy into mass in accordance with Einstein's well-known formula $E = mc^2$. The LHC can produce all known particles, including the top quark, which, with a mass of about 173 GeV, is the heaviest known elementary particle. It was predicted that the SM Higgs boson could also be produced at the LHC if it has a mass less than about 1 TeV.

The SM predicts the cross section for the production of Higgs bosons in proton-proton collisions as a function of its mass. The cross section increases with the center-of-mass energy of the collision and decreases with increasing Higgs mass. Despite the high collision energy, the predicted probability of Higgs boson production is extremely small, about 10^{-10} per collision. Thus, to detect a significant number of Higgs bosons a huge number of collisions must be analyzed, which requires very high luminosity. The maximum instantaneous luminosity achieved so far is $7.6 \times 10^{33} \text{ cm}^{-2} \text{ s}^{-1}$, close to the LHC peak design value that was not expected to be attained until 2015. This was achieved by having 1368 bunches of protons in each beam, spaced 50 ns apart (corresponding to a separation of about 16 m), with each bunch containing about 1.5×10^{11} protons squeezed to a transverse size of about

20 μm at the interaction point. Each bunch crossing yields more than 20 proton-proton collisions on average. The multiple collisions per bunch crossing, known as pileup, are initially registered as a single collision event by the detectors. Resolving the individual collisions within these events is an important challenge for the detectors at the LHC.

The Compact Muon Solenoid (CMS) detector surrounds one of the LHC's interaction points. Heavy particles, such as SM Higgs bosons, created in LHC collisions will typically be unstable and thus rapidly decay into lighter, more stable particles, such as electrons, muons, photons, and hadronic jets (clusters of hadrons travelling in a similar direction). These long-lived particles are what CMS detects and identifies, measuring their energies and momenta with high precision in order to infer the presence of the heavy particles produced in the collisions. Because the CMS detector is nearly hermetic, it also allows for the reconstruction of momentum imbalance in the plane transverse to the beams, which is an important signature for the presence of a neutrino (or a new, electrically neutral, weakly interacting particle) in the collision.

We report the observation of a new particle that has properties consistent with those of the SM Higgs boson. This paper provides an overview of the experiment and results that are described in greater detail in (20). The study examines five SM Higgs boson decay modes. Three modes result in pairs of bosons ($\gamma\gamma$, ZZ , or W^+W^-), and two modes yield pairs of fermions ($b\bar{b}$ or $\tau^+\tau^-$), where γ denotes a photon, Z and W denote the force carriers of the weak interaction, b denotes a bottom quark (and $\bar{b}$ its antiquark), and τ denotes a tau lepton. In the following, we omit the particle charges and use b to refer to both the quark and antiquark. The unstable W , Z , b , and τ particles decay to final states containing electrons, muons, neutrinos, and hadronic jets, all of which can be detected (directly or, in the case of neutrinos, indirectly) and measured with the CMS detector. An independent observation was made by the ATLAS Collaboration experiments (21, 22), which further strengthens our interpretation.

Overview of the CMS detector. The CMS detector measures particles produced in high-energy proton-proton and heavy-ion collisions (23). The central feature of the detector is a superconducting solenoid 13 m long, with an internal diameter of 6 m. Within its volume it generates a uniform 3.8-T magnetic field along the axis of the LHC beams. Within the field volume are a silicon pixel and strip tracker, a lead tungstate (PbWO_4) scintillating crystal electromagnetic calorimeter, and a brass/scintillator hadron calorimeter (HCAL). Muons are identified and measured in gas-ionization detectors embedded in the outer steel magnetic-flux-return yoke. The detector is subdivided into a cylindrical barrel part and endcap disks on each side of the

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The Higgs Boson

CMS DETECTOR

Total weight : 14,000 tonnes
Overall diameter : 15.0 m
Overall length : 28.7 m
Magnetic field : 3.8 T

STEEL RETURN YOKE
12,500 tonnes

SILICON TRACKERS

Pixel (100x150 μm) $\sim 16\text{m}^2 \sim 66\text{M}$ channels
Microstrips (80x180 μm) $\sim 200\text{m}^2 \sim 9.6\text{M}$ channels

SUPERCONDUCTING SOLENOID

Niobium titanium coil carrying $\sim 18,000\text{A}$

MUON CHAMBERS

Barrel: 250 Drift Tube, 480 Resistive Plate Chambers
Endcaps: 468 Cathode Strip, 432 Resistive Plate Chambers

PRESHOWER

Silicon strips $\sim 16\text{m}^2 \sim 137,000$ channels

FORWARD CALORIMETER

Steel + Quartz fibers $\sim 2,000$ Channels

CRYSTAL ELECTROMAGNETIC CALORIMETER (ECAL)

$\sim 76,000$ scintillating PbWO_4 crystals

HADRON CALORIMETER (HCAL)

Brass + Plastic scintillator $\sim 7,000$ channels

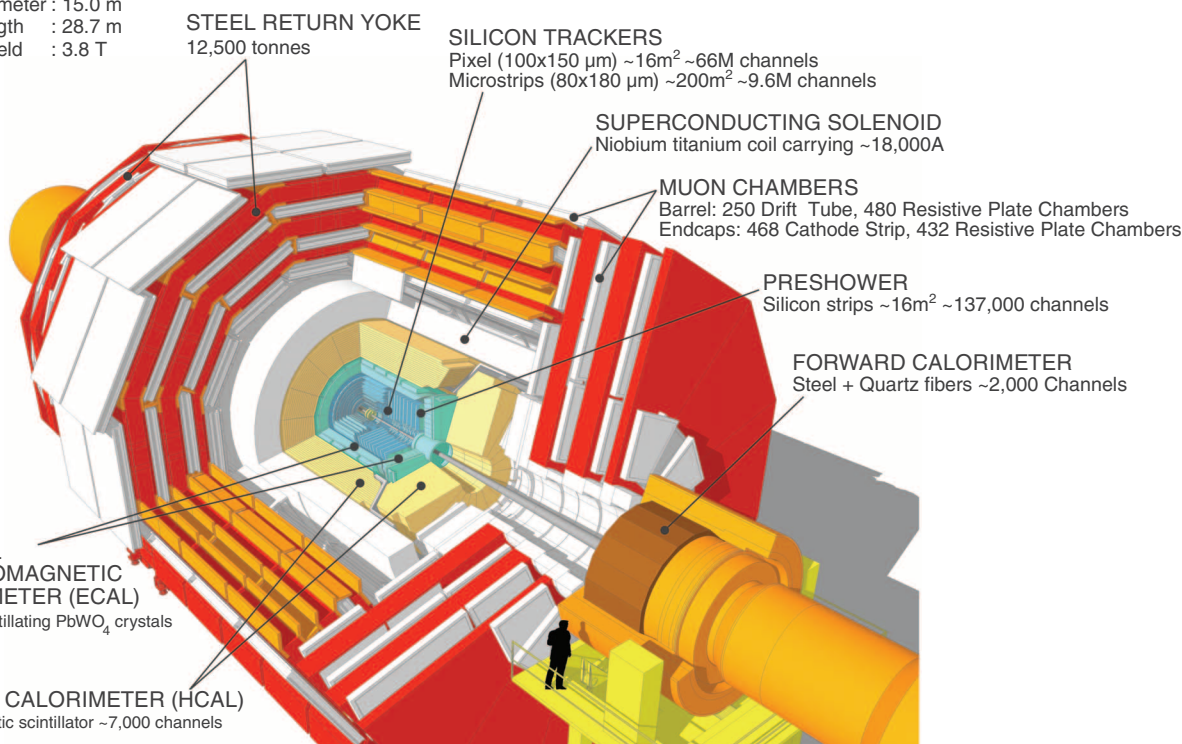
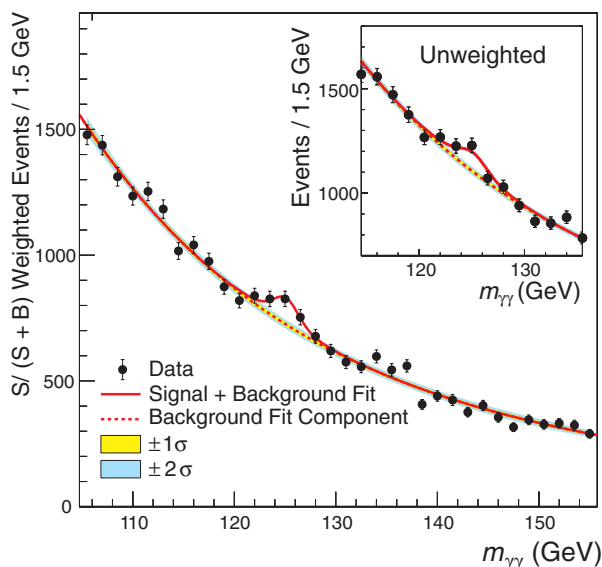


Fig. 1. Schematic view of the CMS detector showing its main components.

Fig. 2. Diphoton ($\gamma\gamma$) invariant mass distribution for the 7- and 8-TeV data collected by CMS in 2011 and 2012, respectively (black points with error bars). The data are weighted by the ratio of the signal to signal plus background for each event class. The solid red line shows the fit result for signal-plus-background; the dashed red line with color bands shows only the background with its uncertainties at 1σ (yellow) or 2σ (cyan). **(Inset)** The central part of the unweighted invariant mass distribution. Integrated luminosity was 5.1 fb^{-1} in 2011 and 5.3 fb^{-1} in 2012.



interaction point. Forward calorimeters complement the coverage provided by the barrel and endcap detectors. The CMS detector has a large angular acceptance, detecting particles over the full azimuthal range and with θ larger than 0.8° , where θ is the polar angle relative to the beam axis. Figure 1 shows the CMS detector and its main components.

The 66 million silicon pixels and 9.3 million silicon strips forming the tracker are used to determine the trajectories of charged particles. The multilayer silicon detectors provide accurate tracking of charged particles with excellent efficiency, which is especially important for the high-pileup conditions at the LHC. The magnetic field curves the trajectories of charged particles,

allowing the measurement of their momenta. The track-finding efficiency is more than 99%, and the uncertainty in the measurement of transverse momentum, p_T (projection of the momentum vector onto the plane perpendicular to the beam axis), is between 1.5 and 3% for charged tracks of $p_T \sim 100\text{ GeV}$. By extrapolating tracks back toward their origins, the precise proton-proton interaction points, or collision vertices, can be determined. Decay vertices of long-lived particles containing heavy-quark flavors, such as B mesons, can similarly be identified and reconstructed. Such “b-tagging” is particularly useful in searches for previously unobserved particles, such as the Higgs boson.

The electromagnetic calorimeter (ECAL) absorbs photons and electrons. These produce showers of particles in the dense crystal material, which yield scintillation light detected by photodetectors glued to the rear faces of the 75,848 crystals. The amount of light detected is proportional to the energy of the incoming electron or photon, allowing their energies to be determined with a precision of about 1% in the region of interest for the analyses reported here. Because electrons are charged particles, they can be discriminated from photons by matching the ECAL signal with a track reconstructed in the tracker.

Hadrons can also initiate showers in the ECAL, but they generally penetrate further into

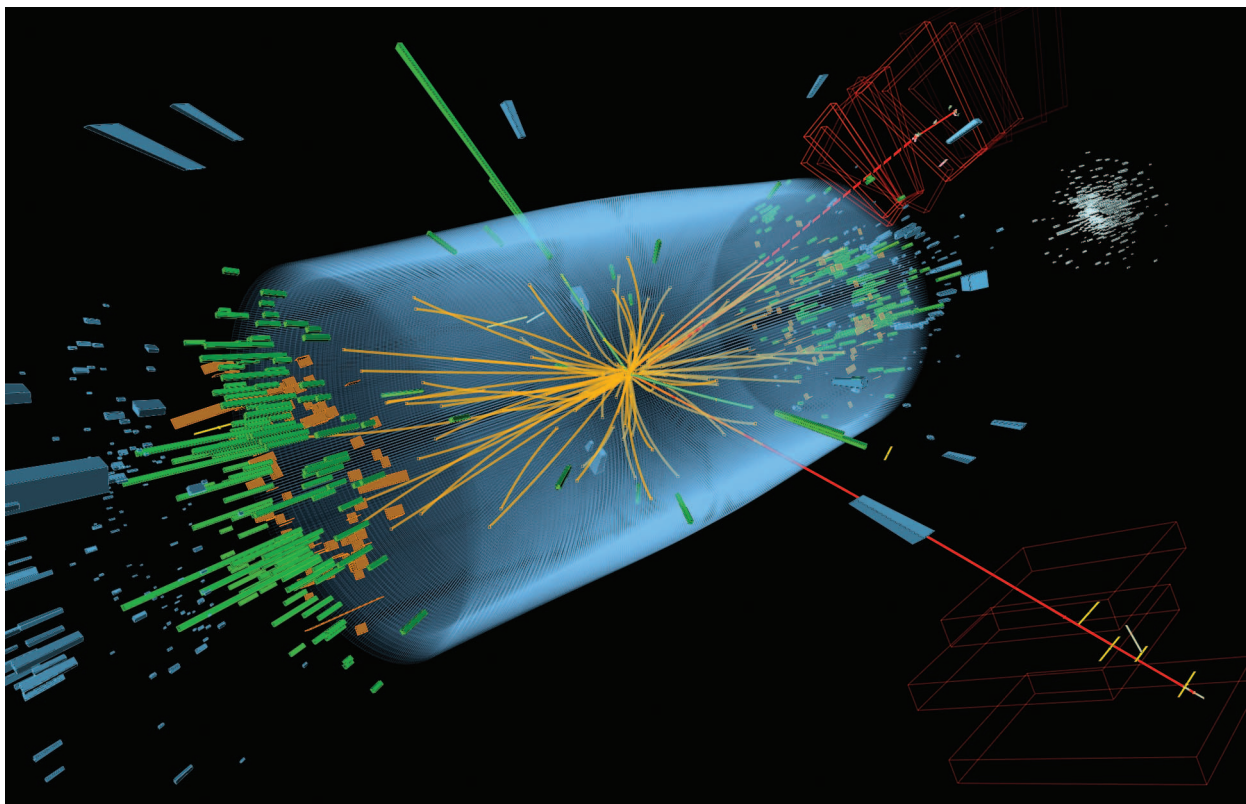


Fig. 3. Event recorded with the CMS detector in 2012 at a proton-proton center-of-mass energy of 8 TeV. The event shows characteristics expected from the decay of the SM Higgs boson to a pair of Z bosons, one of which subsequently decays to a

pair of electrons (green lines in the tracker matched to green towers in the ECAL in the central region of the detector) and the other decays to a pair of muons (red lines). The event could also be due to known SM background processes.

the detector, reaching the HCAL surrounding the ECAL. The measurements of particle energies in the HCAL are not as precise as those of the ECAL but are well adapted to the needs of the CMS physics program.

The solenoid is surrounded by a large detector system that identifies and measures momenta of muons. It comprises three different types of gas-ionization detectors that enable muon momenta to be measured with a precision of less than 1% in the region of interest relevant for the search presented here.

The combination of information from all detectors is used to reconstruct the particle content in a collision event through an algorithm known as particle flow. The quarks and gluons, created in a hard collision of the constituents of the protons, combine and form jets of collimated hadrons in the detector. Once reconstructed from data, the jet energy is calibrated to provide an accurate measurement of the energy of the underlying quark or gluon. A vector sum of the momenta of all visible particles is computed, and the missing transverse momentum deduced from momentum conservation leads to the inference of the presence of undetected particles, such as neutrinos.

Although the LHC typically produces close to half a billion collisions in roughly 20 million

bunch crossings per second, only a tiny fraction of these contain potentially interesting new phenomena, so it is neither necessary nor feasible to record all of the data from every single collision. CMS uses a two-level online trigger system to reduce the event rate from about 20 MHz to about 500 Hz, keeping only those events that are worthy of further investigation. The first level uses custom electronics close to the detector to analyze coarse information from the calorimeters and muon detectors to reduce the rate to 100 kHz or less. The second level uses a computing farm of 13,000 processor cores to analyze the full information from all subdetectors in order to make the final decision on whether to record an event. CMS has thus far selected several billion events, corresponding to more than 4 petabytes of stored event data. The recorded events are sent to computing centers at CERN and around the world to fully reconstruct the particles produced in each collision and allow subsequent analyses.

Searching for the SM Higgs boson. At the LHC, the SM Higgs boson should be produced most efficiently through gluon-gluon fusion: Gluons from each of the colliding protons fuse together to form a Higgs boson. Two additional important production processes are vector boson fusion (VBF), where quarks inside the protons

emit W or Z bosons that fuse to form the Higgs boson, and associated production (VH), where a vector boson V (either a W or Z) is produced together with the Higgs boson. The interacting quarks in the VBF events also give rise to high-energy jets produced at small angles that can be detected and used to help identify this event type. Both VBF and VH events have better signal-to-background ratios relative to gluon fusion but occur far less frequently (24–28).

For every inverse femtobarn ($\text{fb}^{-1} = 10^{-39} \text{ cm}^{-2}$) of integrated luminosity at the LHC, about 20,000 SM Higgs bosons are expected to be produced if the Higgs mass is close to 125 GeV. The majority of these decay to final states that have large backgrounds, making identification difficult or impossible. Dedicated methods have been developed to exploit channels with lower decay fractions by selecting certain kinematical regions of the decay products where the signal-to-background ratio is sufficiently large to make the observation of SM Higgs bosons possible. Extensive use is made of particle-isolation criteria to reject the high-rate jet background, because, in general, the particles from Higgs decays appear relatively isolated from each other and other particles in the detector.

Billions of detailed simulated events have been generated to develop and refine the analysis

techniques needed to estimate the SM Higgs boson signals and backgrounds (29–32). Samples of simulated events reconstructed with the same software as used for the LHC data allow, for example, the estimation of background yields or the prediction of the expected significance for the observation of new particles. However, in the presented analyses the background estimations are derived mostly from the control samples in data.

We studied five SM Higgs decay modes: $H \rightarrow \gamma\gamma$, $H \rightarrow ZZ$, $H \rightarrow WW$, $H \rightarrow \tau\tau$, and $H \rightarrow b\bar{b}$. The $\gamma\gamma$, ZZ , and WW channels are of comparable sensitivity in the search for a Higgs boson with a mass around 125 GeV and are more sensitive than the $b\bar{b}$ and $\tau\tau$ channels. Both the $\gamma\gamma$ and ZZ channels provide precise mass measurements of the parent particle. The presence of an SM Higgs boson decaying to these final states would appear as relatively narrow peaks in the invariant mass spectra of $\gamma\gamma$ and ZZ .

An integrated luminosity of 5.1 fb^{-1} was collected by CMS in 2011 at 7 TeV, allowing a first thorough investigation into the existence, or non-existence, of the SM Higgs boson over a wide mass range. This led to CMS's first significant exclusion of the SM Higgs boson in the medium- and high-mass region between 127 and 600 GeV (33–38). Data from the ATLAS experiment excluded a similar region (39). This left a small window where a low-mass SM Higgs boson could still exist.

In the low-mass region below 127 GeV, the 2011 data analyses also showed an excess over the background-only expectation in the vicinity of 124 GeV. ATLAS observed a similar excess at around the same mass value (39). The observed excess in CMS was inconclusive, being around three standard deviations (3σ) above the background-only expectation. After taking into account the possibility that a signal-like excess could appear randomly in the data between 110 and 145 GeV (the look-elsewhere effect), this significance was reduced to about 2σ . Therefore, there was still a nonnegligible chance that this excess could be due to a random upward fluctuation in the background, making it look like a signal. More data were needed to establish whether this excess was genuine or not. It was predicted that, in the case of a Higgs boson signal, around 10 fb^{-1} more data would be required to reach a statistical significance of around 5σ . However, the LHC operation at 8 TeV in 2012 (giving a 20% increase in Higgs boson production cross section compared to 7 TeV), coupled with improved analyses with 20 to 30% higher sensitivity, reduced this additional required luminosity to around 5 fb^{-1} . By the summer of 2012, CMS had collected an additional 5.3 fb^{-1} of collision data at this new energy.

Because the 2011 analysis (33–38) showed an excess of events at about 125 GeV and to avoid a potential bias in the choice of selection

criteria for the 2012 data that might artificially enhance this excess, we performed the analysis of the 2012 data “blind”: The region where the signal may be present was not examined until after all the analysis criteria had been fully scrutinized and agreed upon within the collaboration.

Search for the SM Higgs boson decay into two photons. The predicted probability for a 125-GeV SM Higgs boson to decay into two photons is about 0.3%. Yet this decay mode is one of the most important, because both photons can be measured very accurately in the CMS ECAL and the backgrounds can be precisely estimated. The presence of a signal would manifest itself as a narrow peak above a smoothly falling background in the invariant mass distribution of the two photons.

The energy resolution and precise knowledge of the absolute energy scale of the ECAL are key elements of this analysis. These were achieved by calibrating each channel of the ECAL in situ, using diphoton decays of π^0 and η^0 , for example. The stability of the ECAL response was ensured by the use of a sophisticated real-time monitoring procedure that corrects any deviations with a precision of a few per mill. Decays of Z bosons into electron pairs were then used to determine the energy resolution and energy scale, taking advantage of the precise knowledge of the Z mass and width.

An additional challenge is to determine from which of the many collision vertices in the event the two photons originate, which affects the precision of the mass measurement of the parent particle. The collisions occurring in a single LHC bunch crossing, as many as 40 in 2012, are spread over a distance of about 10 cm along the beam axis at the center of CMS. Because photons do not leave tracks in the detector, there can be ambiguity as to which collision vertex they belong to. A variety of techniques were used to deter-

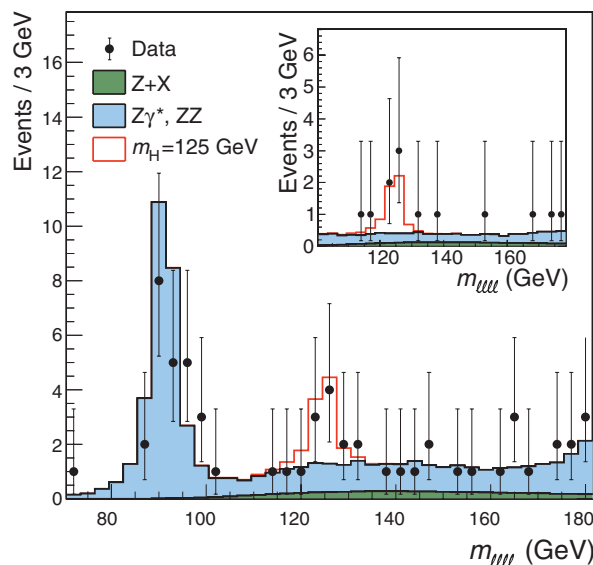
mine the diphoton vertex, including use of event kinematics and an understanding of photon conversions into electron pairs.

Multivariate analyses (40, 41) based on boosted decision trees were used to identify the photons and to extract their energies and uncertainties on a photon-by-photon basis. To optimize sensitivity, we categorized diphoton events into four classes with decreasing restrictions on the quality according to many variables, including the uncertainty on the diphoton mass measurement, the kinematics of the photons, and whether the photons convert into electron pairs in the material before reaching the ECAL. For example, events where both photons are in the central region of CMS and do not convert into electron pairs in the tracker were given the highest classification because they are the most precisely measured. We also included additional classes for diphoton events that have two additional jets with properties consistent with those expected for the VBF production process.

Photons of high quality (determined from the spatial distribution of electromagnetic showers and isolation criteria) were selected with energies above 30 to 40 GeV, depending on the event class. Figure 2 shows the diphoton invariant mass spectrum from all the data collected by CMS from 2011 to mid-2012, after selections as defined in (20). The spectrum was built up from the event classes, with each class weighted by the ratio of the signal to signal-plus-background estimated from simulation. An excess was observed at 125 GeV on an otherwise smoothly falling background spectrum. The background consists mostly of collisions where two photons are produced in SM processes and a smaller fraction from events where at least one of the photon signals is not genuine but originates from the debris of jets.

The observed excess is consistent in shape and size with that expected for diphoton decays

Fig. 4. Distribution of the four-lepton reconstructed invariant mass for the sum of the $4e$, 4μ , and $2e2\mu$ channels. Points represent the data, shaded histograms represent the background, and the empty histogram the signal expectations. The distributions are presented as stacked histograms. The measurements are presented for the sum of the data collected at center-of-mass energies of 7 and 8 TeV during 2011 and 2012, respectively. Error bars represent standard deviations. (Inset) The four-lepton invariant mass distribution after selection of events with signal-like kinematics, as described in the text.



of SM Higgs bosons. To evaluate the significance of the signal, we fitted the background spectrum over the whole mass range with a fifth-order polynomial function (or a third-order polynomial for the VBF categories) and measured the magnitude of the excess above the background. The diphoton decay mode has a signal significance of 4.1σ relative to the background-only hypothesis. This excess is present in both 2011 and 2012 data and is consistent between the two data sets.

The observation of the diphoton final state also implies that the new particle is a boson and has an integer spin different from unity (42, 43).

Search for the SM Higgs boson decay into two Z bosons. If the SM Higgs boson has a mass of 125 GeV, about 2.6% of them are predicted to decay into two Z bosons. At least one of the Z bosons is necessarily virtual, that is, it has a different mass than the 91 GeV Z mass. The Z bosons each decay into pairs of leptons or quarks. We concentrate on the Z decays into leptons, particularly electrons (e) and/or muons (μ), because these have the smallest SM backgrounds. In CMS, we analyzed separately the three different final states in this channel, namely $2e2\mu$, $4e$, and 4μ , and then combined the results.

The invariant mass of the ZZ system can be reconstructed and measured with good accuracy in CMS from the four-lepton momenta. Hence, the presence of a Higgs boson in the data should manifest itself as a peak in the ZZ invariant mass spectrum in the presence of a small continuum background.

There are numerous SM processes (not including Higgs boson decays) that can lead to the same final states. They include direct ZZ production from quark-antiquark annihilation and gluon-gluon fusion, as well as processes involving a single Z boson produced with associated heavy-quark jets and top-antitop pair-production. Apart

from the rate of direct ZZ production, which we can determine accurately from simulation, the rates of other backgrounds were extracted from data.

The leptons from Z decays are, in general, well isolated in the detector; that is, their trajectories are far from the debris of jets or other particles produced in the collision. Despite the large particle multiplicity per event from pileup interactions, the overall efficiency for selecting isolated leptons remains very high.

We selected collisions with four isolated leptons originating from the same vertex, for which the transverse momentum of each muon is at least 5 GeV and of each electron is at least 7 GeV. (These criteria were determined by using a large sample of single-Z events collected in the past 2 years.) Both Z boson candidates are required to decay to two same-flavor leptons of opposite charge, and the invariant mass of the dileptons produced in the Z boson decays must be in the range from 40 to 120 GeV for the heavier of the two and 12 to 120 GeV for the lighter one.

Figure 3 shows a typical event containing two reconstructed Z bosons, with a ZZ invariant mass around 125 GeV. The ZZ invariant mass spectrum for selected events is shown in Fig. 4. Because leptons (especially electrons) can often radiate an energetic photon at an early stage of their trajectory through the detector, the energy or momentum of such leptons can be considerably underestimated. We therefore searched for energetic photons close to these leptons and added their energies when appropriate.

The invariant mass spectrum in Fig. 4 shows a Z peak at 91 GeV resulting from decays of Z bosons into two leptons and an energetic virtual photon that materializes through a second dilepton pair. There is also a statistically significant peak near 125 GeV. This completely independent analysis indicates the presence of a signal in the same region as that found in the diphoton

decay mode. This is to be expected if indeed the signals correspond to the same parent particle.

The signal-to-background separation improves further by exploiting the decay kinematics expected for signal events, especially the decay angles and invariant masses of the two pairs of leptons (44). Analyzing events in the peak at 125 GeV confirmed that many of these events have the requisite characteristics; this reinforced our interpretation that the signal is genuine. The statistical significance of the excess observed by combining data from 2011 and 2012, accounting also for the decay-angle characteristics, is 3.2σ relative to the background-only hypothesis. The maximum significance occurs at a mass of 125.6 GeV.

Search for Higgs boson decays in other channels. Apart from the $\gamma\gamma$ and ZZ channels discussed above, CMS also searched for decays of SM Higgs bosons to two W bosons, two τ leptons, or two b quarks.

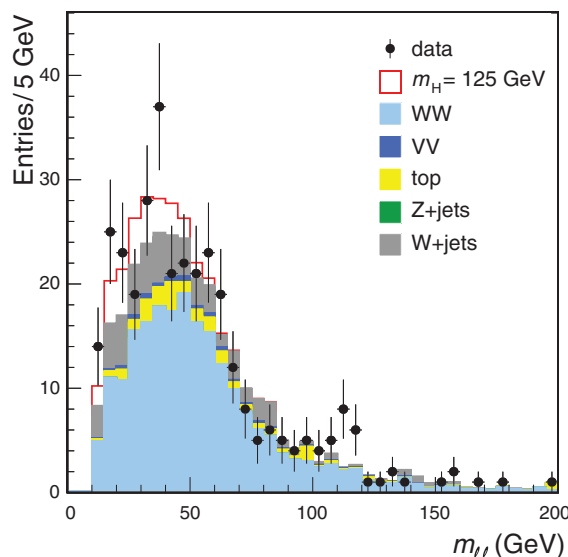
For the WW decay mode, the final states must contain two opposite-sign leptons (either electrons or muons) and significant missing transverse momentum, resulting from the undetected neutrinos from W decays. In contrast to the $\gamma\gamma$ and ZZ modes, the invariant mass of the two W bosons cannot be precisely reconstructed. The potential excess in the data over the background expectation provides only a continuum instead of a sharp resonance peak. We used multivariate analysis techniques to optimize the sensitivity to a possible signal present in data. We classified the events into a number of exclusive categories, for example, according to lepton flavor content and whether there are jets present (to enhance VBF production relative to gluon fusion).

These different event classes were subject to different backgrounds and have different sensitivities. The challenge for this analysis was to estimate the backgrounds from the SM, which was generally achieved through techniques based on control regions in the data and complemented through simulation. Special attention was paid to determining the missing transverse momentum, particularly in the presence of large pileup (as in the 2012 data sample).

We selected events in which the p_T of the most energetic lepton is greater than 20 GeV and that of the second-most-energetic lepton is above 10 GeV and that have missing transverse momentum typically above 20 GeV. The results of this analysis, combining all the classes across the 2011 and 2012 data, show (Fig. 5) a broad excess of events over the expected background, consistent with the presence of a new particle at a mass near 125 GeV. The statistical significance is about 1.5 to 2.0σ relative to the background-only hypothesis.

We also explored whether this new particle decays into fermion pairs, as it would be expected to if the associated field gives mass to the fermions in addition to the W and Z bosons, by

Fig. 5. Distribution of the invariant mass of lepton pairs for the zero-jet $e\mu$ category in the search at 8 TeV for the SM Higgs boson decay to a pair of W bosons. The signal expected from the SM Higgs boson with a mass $m_H = 125$ GeV is shown added to the background. Error bars indicate standard deviations.



The Higgs Boson

looking for instances where the particle decays into heavy fermions. The heaviest fermions into which a 125-GeV SM Higgs boson can decay are the τ leptons and b quarks.

The detection of τ leptons is challenging because they are unstable and decay less than 10^{-12} s after production, either into a lighter charged lepton (electron or muon) and neutrinos or into a neutrino and either one or three charged pions possibly accompanied by neutral pions. CMS has tools to detect and reconstruct such decays and separate these from backgrounds. Several decay channels were explored, including the combination of one τ lepton decaying exclusively into leptons and the other into hadrons. A natural process to calibrate the analysis is through Z boson production, where 3.4% of the Z decays are into $\tau^+\tau^-$ pairs, and CMS has successfully used this decay channel (45).

The main challenge for this search is to assess the backgrounds, most of which are extracted using control samples in data. Different τ decay channels are analyzed separately and classified accordingly, including classes with accompanying jets. The results of these individual analyses were then combined for a final result.

As in the case of the WW decay mode, the presence of neutrinos in the decay products of the τ leptons prevents a full event reconstruction, and, instead of a resonance peak, a broad enhancement over background is expected. We have not yet found such an enhancement, but the current sensitivity to this channel does not exclude the presence of the SM Higgs boson. With the LHC on course to triple the integrated luminosity by the end of 2012, studies of the $\tau\tau$ channel will become more sensitive.

Lastly, CMS conducted a search for SM Higgs bosons decaying into two b quarks. Each quark gives rise to a jet that is recognized in the analysis ("tagged") as originating from b quarks. For the tagging of b quarks, we searched for secondary vertices in the jets, caused by decays of B hadrons that travel a few millimeters before decaying. The energy of the original b quark is estimated from the energies of all the particles in its jet and has a large uncertainty. The reconstructed masses of objects obtained from these jets are therefore expected to be distributed over a region of about 20 GeV in the mass range of interest.

At low mass (below about 135 GeV), the SM Higgs boson decay into b quarks has the largest rate of the five search modes we report in this paper, and we therefore expect a large number of such decays in the data. This signal is, however, overwhelmed by a large background from SM b quark production, making the search less sensitive. To have a more favorable signal-to-background ratio, we searched for this signal in the (rarer) associated production process involving a W or Z boson, which can be detected from their leptonic decays. We required these bosons to have transverse momenta above 50 GeV.

To minimize the background in the bb channel, we again used several mutually exclusive classes of events, which were analyzed separately. These classes are based on the transverse momentum of the jet pair and the nature and decay of the associated boson. For the final result, we combined all these channels and used all of the available data from 2011 and 2012. The result shows a small excess above the background-only expectation over a large mass range, including the region near 125 GeV. The sensitivity of this analysis is about 1.5 times lower than required for concluding whether a signal is present (as expected from SM prediction) or if the coupling to b quarks is different from what we would expect. Again, tripling the amount of collision data should be decisive.

In conclusion, neither fermion decay mode shows, at present, a statistically significant enhancement over the background-only expectation.

Nevertheless, at the present level of sensitivity the results in these channels are consistent with the production of the SM Higgs boson, in agreement with observations in the other three (diboson) decay modes.

Observation of a new particle. The final result combines all the information collected through a global fit (46) to the five different search channels. The result reflects the probability for the background to deviate from the expectation by at least the observed amount, assuming the absence of the SM Higgs boson in this mass range. This probability, known as the local P value, is evaluated by using sets of simulated data that incorporate all experimental uncertainties and correlations among analyses. The result is shown (Fig. 6) for each of the five search channels individually, as well as for the combination of all five channels. For the combination the minimum P value at 125 GeV is of the order of one in three million.

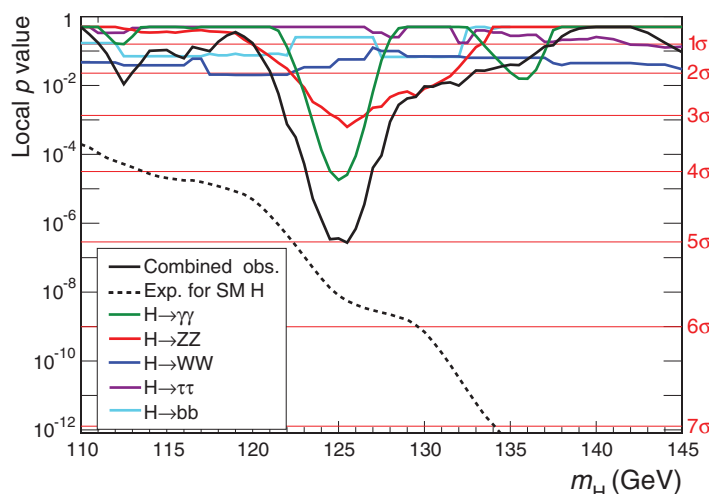


Fig. 6. The observed probability (local P value) that the background-only hypothesis would yield as many (or more) events as seen in the CMS data, as a function of the mass of the SM Higgs boson for each of the five search channels individually and the combination of all five channels. The dashed line shows the median expected local P values for the SM Higgs boson with a mass m_H .

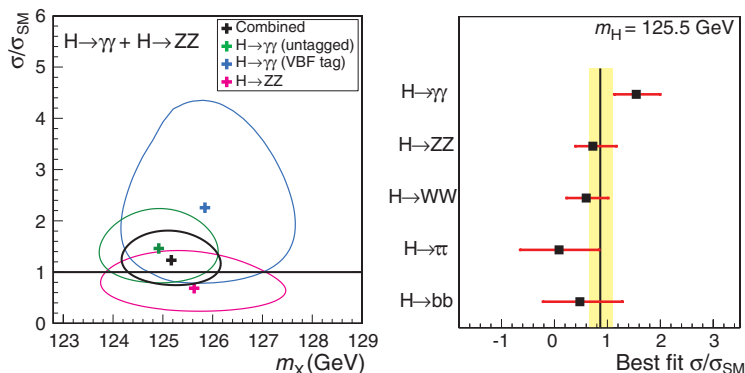


Fig. 7. (Left) 68% CL contours for the relative signal strength σ/σ_{SM} versus the Higgs boson mass for the high-precision decay modes ($\gamma\gamma$ and ZZ). **(Right)** The relative signal strength σ/σ_{SM} for the five Higgs boson decay modes examined by CMS. The symbol σ/σ_{SM} denotes the production cross section multiplied by the relevant branching fraction, relative to the SM expectation.

This probability corresponds to a local significance of 5σ . The probability of observing this large a fluctuation anywhere in the mass range of 114 to 130 GeV, where the Higgs boson had not been excluded by previous data, is small and results in a global significance of 4.6σ . The global significance is smaller than the local value because of the look-elsewhere effect. Both measures convincingly show that this is not a background fluctuation, but rather the observation of a new particle. The expected sensitivity with the present data for a 125 GeV SM Higgs boson amounts to a local significance of $5.8 \pm 1.0\sigma$, consistent with the signal observed at 5σ .

In addition to being able to say with high confidence that a new particle has been observed, and that it is a boson with spin not equal to one, we were also able to derive some of its properties, such as its mass. And, as mentioned above, once the mass is known the SM allows us to calculate many other properties, such as the fractions of Higgs bosons decaying in different ways, and compare these expectations with our measurements. This is expressed as the signal strength, that is, the measured production rate of the signal, which can be determined for each decay mode individually and for the overall combination of all channels, normalized to the predicted Higgs boson production rate. The signal strength was defined to be equal to one for the SM Higgs boson. The measured signal strength was highest in the diphoton channel, namely 1.6 ± 0.4 , whereas that in the ZZ channel was $0.7^{+0.4}_{-0.3}$. By using the high-resolution diphoton and ZZ channels discussed above, which show a resonance peak, we obtained the 68% confidence level (CL) contours for the signal strength versus the boson mass (Fig. 7 left). We also show the combination of the diphoton and ZZ decay modes, where the relative signal strengths of these two modes are constrained by the expectations for the SM Higgs boson. To extract the value of the mass in a model-independent way, we allowed the signal yields of the combined channels to vary independently. The combined best-fit mass is 125.3 ± 0.4 (statistical) ± 0.5 (systematic) GeV.

The signal strengths for all five channels are depicted in Fig. 7 (right). The overall combined signal strength, including all channels, is 0.87 ± 0.23 . Hence, these results are consistent, within relatively large statistical and systematic uncertainties, with the expectations for the SM Higgs boson.

The CMS data also rule out the existence of the SM Higgs boson in the ranges of 114.4 to 121.5 GeV and 128 to 600 GeV at 95% CL (20). Lower masses were already excluded by CERN's Large Electron Positron collider at the same CL (14).

More data are needed to establish whether this new particle has all the properties of the SM Higgs boson or whether some do not match. The latter may imply new physics beyond the

SM. This particle has the potential to be a portal to a new landscape of physical phenomena that is still hidden from us. The CMS experiment is in an excellent position to undertake this research in the years to come.

References and Notes

1. S. L. Glashow, *Nucl. Phys.* **22**, 579 (1961).
2. S. Weinberg, *Phys. Rev. Lett.* **19**, 1264 (1967).
3. A. Salam, in *Elementary Particle Physics: Relativistic Groups and Analyticity*, N. Svartholm, Ed. (Nobel Symposium 8, Almqvist and Wiskell, Stockholm, 1968), pp. 367–377.
4. F. Englert, R. Brout, *Phys. Rev. Lett.* **13**, 321 (1964).
5. P. W. Higgs, *Phys. Lett.* **12**, 132 (1964).
6. P. W. Higgs, *Phys. Rev. Lett.* **13**, 508 (1964).
7. G. S. Guralnik, C. R. Hagen, T. W. B. Kibble, *Phys. Rev. Lett.* **13**, 585 (1964).
8. P. W. Higgs, *Phys. Rev.* **145**, 1156 (1966).
9. T. W. B. Kibble, *Phys. Rev.* **155**, 1554 (1967).
10. J. M. Cornwall, D. N. Levin, G. Tiktopoulos, *Phys. Rev. Lett.* **30**, 1268 (1973).
11. J. M. Cornwall, D. N. Levin, G. Tiktopoulos, *Phys. Rev. D* **10**, 1145 (1974).
12. C. H. Llewellyn Smith, *Phys. Lett. B* **46**, 233 (1973).
13. B. W. Lee, C. Quigg, H. B. Thacker, *Phys. Rev. D Part. Fields* **16**, 1519 (1977).
14. ALEPH, DELPHI, L3, OPAL Collaborations, and LEP Working Group for Higgs Boson Searches, *Phys. Lett. B* **565**, 61 (2003).
15. T. Aaltonen *et al.*; CDF Collaboration; D0 Collaboration, *Phys. Rev. Lett.* **104**, 061802 (2010).
16. CDF Collaboration, "Combined search for the standard model Higgs boson decaying to a bb pair using the full CDF data set" (2012), <http://arxiv.org/abs/1207.1707>.
17. T. Aaltonen *et al.*; CDF Collaboration; D0 Collaboration, *Phys. Rev. Lett.* **109**, 071804 (2012).
18. D0 Collaboration, "Combined search for the standard model Higgs boson decaying to bb using the D0 Run II data set" (2012), <http://arxiv.org/abs/1207.6631>.
19. L. Evans, P. Bryant, *J. Instrum.* **3**, S08001 (2008).
20. CMS Collaboration, *Phys. Lett. B* **716**, 30 (2012).
21. ATLAS Collaboration, *Phys. Lett. B* **716**, 1 (2012).
22. ATLAS Collaboration, *Science* **338**, 1576 (2012).
23. CMS Collaboration *et al.*, *J. Instrum.* **3**, S08004 (2008).
24. J. R. Ellis, M. K. Gaillard, D. V. Nanopoulos, *Nucl. Phys. B* **106**, 292 (1976).
25. H. M. Georgi, S. L. Glashow, M. E. Machacek, D. V. Nanopoulos, *Phys. Rev. Lett.* **40**, 692 (1978).
26. S. L. Glashow, D. V. Nanopoulos, A. Yildiz, *Phys. Rev. D* **18**, 1724 (1978).
27. S. Alioli, P. Nason, C. Oleari, E. Re, *J. High Energy Phys.* **0904**, 002 (2009).
28. P. Nason, C. Oleari, *J. High Energy Phys.* **1002**, 37 (2010).
29. T. Sjöstrand, S. Mrenna, P. Z. Skands, *J. High Energy Phys.* **0605**, 026 (2006).
30. S. Gieseke *et al.*, "Herwig++ 2.0 Release Note" (2006), <http://arxiv.org/abs/hep-ph/0609306>.
31. J. Alwall *et al.*, *J. High Energy Phys.* **0709**, 028 (2007).
32. S. Agostinelli *et al.*, *Nucl. Instrum. Meth. A* **506**, 250 (2003).
33. CMS Collaboration, *Phys. Lett. B* **710**, 26 (2012).
34. CMS Collaboration, *Phys. Lett. B* **710**, 403 (2012).
35. CMS Collaboration, *Phys. Rev. Lett.* **108**, 111804 (2012).
36. CMS Collaboration, *Phys. Lett. B* **710**, 91 (2012).
37. CMS Collaboration, *Phys. Lett. B* **713**, 68 (2012).
38. CMS Collaboration, *Phys. Lett. B* **710**, 284 (2012).
39. ATLAS Collaboration, *Phys. Rev. D* **86**, 032003 (2012).
40. H. B. Prosper, paper presented at XII International Workshop on Advanced Computing and Analysis Techniques in Physics Research (ACAT08), 3 to 7 November 2008, Erice, Italy, no. PoS(ACAT08)010.
41. P. C. Bhat, *Annu. Rev. Nucl. Part. Sci.* **61**, 281 (2011).
42. L. D. Landau, *Dokl. Akad. Nauk* **60**, 207 (1948).
43. C. N. Yang, *Phys. Rev.* **77**, 242 (1950).
44. CMS Collaboration, *J. High Energy Phys.* **4**, 36 (2012).
45. CMS Collaboration, *J. High Energy Phys.* **08**, 117 (2011).
46. ATLAS and CMS Collaborations, technical report ATL-PHYS-PUB 2011-11, CMS NOTE 2011/005 (2011), <http://cdsweb.cern.ch/record/1379837>.

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Supplementary Materials

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A Particle Consistent with the Higgs Boson Observed with the ATLAS Detector at the Large Hadron Collider

The ATLAS Collaboration*†

Nearly 50 years ago, theoretical physicists proposed that a field permeates the universe and gives energy to the vacuum. This field was required to explain why some, but not all, fundamental particles have mass. Numerous precision measurements during recent decades have provided indirect support for the existence of this field, but one crucial prediction of this theory has remained unconfirmed despite 30 years of experimental searches: the existence of a massive particle, the standard model Higgs boson. The ATLAS experiment at the Large Hadron Collider at CERN has now observed the production of a new particle with a mass of 126 giga-electron volts and decay signatures consistent with those expected for the Higgs particle. This result is strong support for the standard model of particle physics, including the presence of this vacuum field. The existence and properties of the newly discovered particle may also have consequences beyond the standard model itself.

The standard model (SM) of particle physics (1–4) describes the fundamental particles and the electromagnetic, weak, and strong forces between them. It has been extremely successful at describing experimental data and predicting new results since its proposal in the 1960s. In the SM, forces are mediated by the exchange of particles with spin, which are known as bosons. These bosons are exchanged between electromagnetic, weak, and strong charges. The charges are carried by the fundamental constituents of matter: six quarks and six leptons, and their antiparticles, together known as fermions. The photon (γ), the boson that mediates electromagnetism, and gluons, the bosons that mediate the strong force, are massless. However, the carriers of the weak force, the W and Z bosons—which are responsible for, for example, radioactivity and hydrogen fusion in the Sun—are observed to have masses ~ 100 times that of the proton. In the SM, the W and Z bosons obtain their mass through their interactions with a field of weak charge that is postulated to pervade the vacuum (5–10) of space.

A critical prediction of the SM is that if enough energy is available, excitation of this vacuum field will produce a massive particle with zero spin: the Higgs boson, commonly denoted H . The Higgs boson is fleeting; it is predicted to decay rapidly to other particles. Although many searches for the Higgs boson have been carried out since its prediction, it has remained elusive. The mass of the Higgs boson, m_H , is not specified by the SM. Quantum mechanical effects link m_H to properties of known particles such as the W

boson and the t quark; the results of decades of precision measurements of such properties (11–13) indicate that m_H is 94^{+29}_{-24} GeV, but only at the 68% confidence level (CL) (14). This is slightly above the masses of the W and Z bosons. About 10 years ago, searches at the CERN Large Electron Positron (LEP) collider indicated that m_H was greater than 114.4 GeV at the 95% CL (15). After 25 years of collecting data, experiments at the Tevatron proton-antiproton collider at Fermilab recently excluded the mass region 147 to 180 GeV at the 95% CL (16).

One of the main goals of the Large Hadron Collider (LHC) (17) physics program is to test this critical prediction of the SM: to either observe the SM Higgs boson and measure its properties or disprove its existence. The LHC accelerates two counter-rotating proton beams to nearly the speed of light so that the energy upon their collision should be sufficient to produce Higgs bosons over their expected mass range. The challenge is resolving the rare signal of the Higgs boson among a huge background of similar particles produced by the energetic collisions. Detection of a Higgs boson requires computing its mass from the total energy and momentum of all its decay particles. Unstable particles, such as W and Z bosons, may also be produced as intermediate quantum states with invariant masses well below their nominal masses.

Guided by our knowledge of the detector response to particles, we selected samples of events that the SM predicts to be enriched with Higgs bosons from the various decay channels. A Higgs boson with a mass of ~ 126 GeV would have five main experimentally accessible decay channels ($H \rightarrow \gamma\gamma$, ZZ , WW , bb , or $\tau\tau$), where b denotes a b quark, and τ denotes the heaviest lepton (tau). Other SM processes contribute to the background. Evidence for Higgs boson production is inferred

from statistically significant excesses of events above the background predictions.

The LHC includes two detectors specifically designed for this search. In 2011, the LHC operated with a total proton-proton collision energy of 7 TeV. Both the A Toroidal LHC Apparatus (ATLAS) Collaboration (18) and the Compact Muon Solenoid (CMS) Collaboration experiments (19) ruled out SM Higgs boson production in most of the remaining mass region considered relevant, from 110 to 600 GeV. However, the two experiments observed tantalizing hints of a new particle with mass in the region 124 to 126 GeV and compatible with a SM Higgs boson (20, 21).

The LHC's collision energy was raised to 8 TeV in 2012, increasing the predicted production rate of SM Higgs bosons and the sensitivity of the search. In order to avoid observer bias, all details of the analyses, such as the set of search channels, the event selection criteria, and the signal and background predictions, were fixed before examining the signal regions of the April–June 2012 data. Here, we report the discovery of a SM Higgs-like boson in the combined data collected with the ATLAS detector during 2011 and April–June 2012. This paper provides an overview of the experimental results that are described in more detail in (22). Independently, the CMS experiment also identified a similar boson at the same mass, as discussed in (23, 24).

The ATLAS detector. The design of the cylindrically symmetric ATLAS detector (18) was optimized to study a broad range of physics processes, including SM Higgs boson production, over a wide mass range. The entire detector (Fig. 1) weighs 7000 metric tons. It is 44 m long and 25 m in diameter. It is located in an underground cavern at a depth of 100 m, where it surrounds one of the collision points around the 27-km-long LHC ring. ATLAS is actually composed of several distinct subdetectors in order to identify and measure the energy and momentum of a variety of particles and so reconstruct the dynamics of the collision.

The momenta of charged particles are measured by an inner tracking detector (ID) immersed in a 2-T axial field provided by a superconducting magnet. The energies of electrons and photons are measured in an electromagnetic calorimeter (ECAL) that surrounds the inner detector and magnet. An additional layer of calorimeters outside the ECAL for measuring hadrons (such as protons and neutrons) also serves as an absorber, so that only energetic muons and the elusive weakly interacting neutrinos penetrate it. The muon spectrometer surrounds the calorimeters; it consists of superconducting magnets providing a toroidal field and a system of precision charged-particle detectors.

The combination of the subdetectors provides particle energy and momentum measurements, together with electron, muon, and photon identification, over more than 98% of the solid angle. The measurements are made by ~ 90 million sensor elements, most of which are in the inner de-

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tector. Jets (narrow cones of particles produced by the conversion of quarks and gluons to hadrons) are reconstructed by using the nearly 4π solid angle coverage of the calorimeters. In high-energy proton-proton collisions, only one constituent (a quark or a gluon) from each proton takes part in the interactions that result in a wide variety of known and possibly unknown processes, including production of the SM Higgs boson. The remnants of two colliding protons tend to travel along the beam directions and exit the detector unobserved, so it is only possible to study momentum balance in the plane transverse to the proton beam axis. Neutrinos, which are normally not directly detectable, are inferred from their transverse momenta; they are assumed to balance the sum of the transverse momenta of the observed electrons, muons, photons, and jets: Their presence is thus indicated by the magnitude of the missing transverse momentum, denoted E_T^{miss} .

During standard LHC operation, two counter-rotating packets of protons cross at the center of ATLAS every 50 ns. The high intensity of the proton beams results in multiple proton-proton collisions occurring during each crossing of proton packets, an effect known as “pile-up” (Fig. 2). The average number of interactions per proton-packet crossing was ~ 10 in 2011; it increased to ~ 20 in 2012, but advances in understanding the detector performance and improved analysis techniques mitigate the effects of the harsher environment.

The set of digitized signals recording the collision products of a single crossing of proton pack-

ets is known as an event. A three-level trigger system decides which events should be recorded; typically, 20 potentially interesting events are selected out of 1 million produced. Each trigger level reduces the rate by a factor between 10 and 100. In this way, only the most interesting events (those with high transverse momentum electrons, muons, photons, or jets) are recognized and recorded. Each event requires ~ 1 megabyte of storage, and typically 400 events are recorded every second.

Further details of the design of the detector are given in (25).

Signal expectation and background estimation. The most important SM Higgs boson production process in the energy range of the LHC is expected to be gluon fusion. Gluons do not directly produce the SM Higgs boson but rather do so indirectly through a quantum loop process involving mainly the heaviest (t) quark (Fig. 3). Other processes are predicted to provide much clearer signals but at substantially reduced rates. The production and decay rates used to infer signal yields in our analysis are taken from theoretical predictions (26, 27).

The background rates and signal efficiencies are estimated from the data as far as possible. To supplement this, we simulated the production of the SM Higgs boson signal and relevant background processes. These simulations use mathematical functions, constrained by experimental data, to describe the energy distributions of quarks and gluons in the colliding protons and how they interact and describe in detail how the outgoing

particles behave in the ATLAS detector (28, 29). They also include modeling of the pile-up conditions observed in the data.

The SM predicts that $\sim 200,000$ SM Higgs bosons are produced in the combined ATLAS data if m_H is 126 GeV. However, because most of the decays are indistinguishable from the 8×10^{14} inelastic proton-proton collisions in the combined ATLAS data, we focused on a few distinctive SM Higgs boson decay modes. Two of the most sensitive channels are the decay into two photons (denoted the $H \rightarrow \gamma\gamma$ channel) and the decay into two Z bosons, which in turn each decay into an oppositely charged pair of electrons or muons [denoted the $H \rightarrow ZZ \rightarrow llll$ channel (30)]. Both of these channels were examined in the data from 2011 and 2012. An additional sensitive decay mode involving two W bosons decaying to an electron, a muon, and two neutrinos (denoted the $H \rightarrow WW \rightarrow e\mu\nu\nu$ channel) was included in the 2012 search. Additional channels in which the SM Higgs boson decays to pairs of b quarks or τ leptons, or alternative decay patterns for the W or Z bosons, have so far been studied in 2011 data only because it takes more time to study their more complex signatures.

$H \rightarrow \gamma\gamma$ channel. The decay of the SM Higgs boson to a pair of photons proceeds mainly via quantum loop processes involving the W boson, as illustrated in Fig. 4. The fraction decaying in this way is never large, typically 0.2%; however, the signature of two high-energy photons isolated from any other sizable activity in the

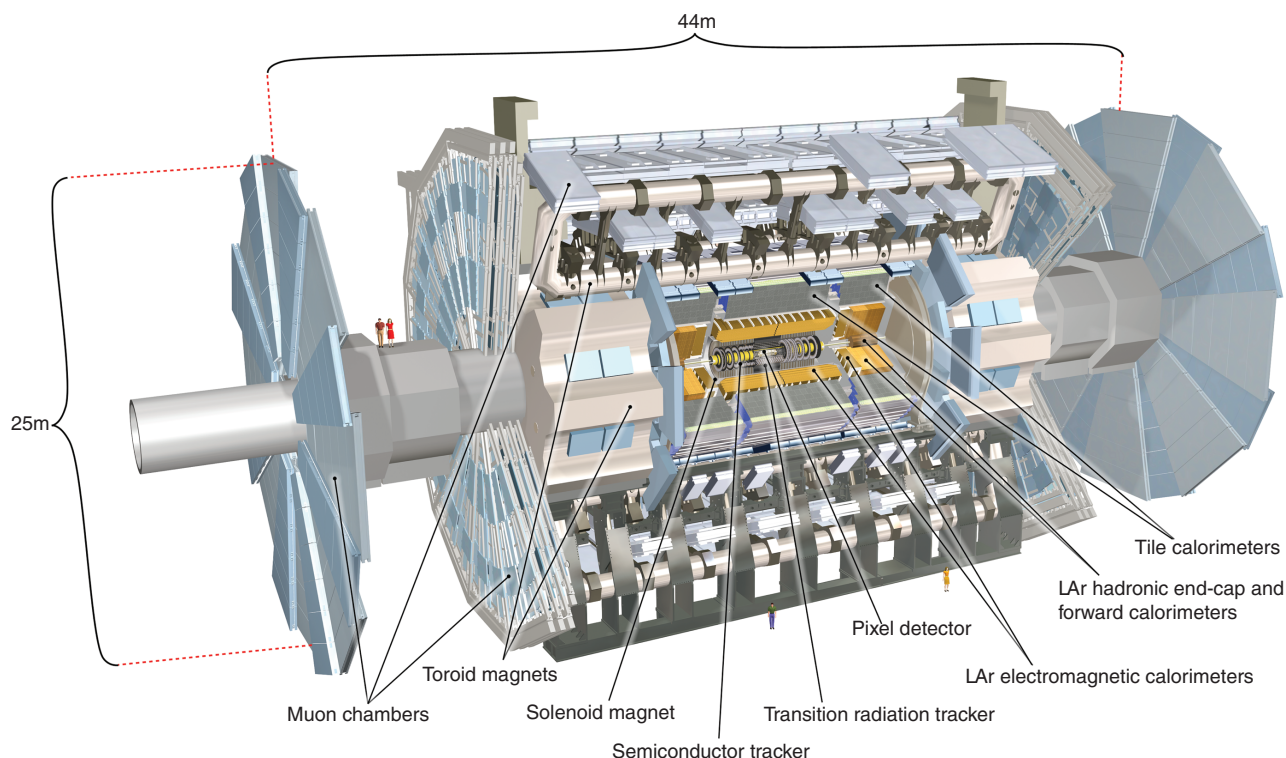


Fig. 1. Cutaway drawing of the ATLAS detector showing its main components.

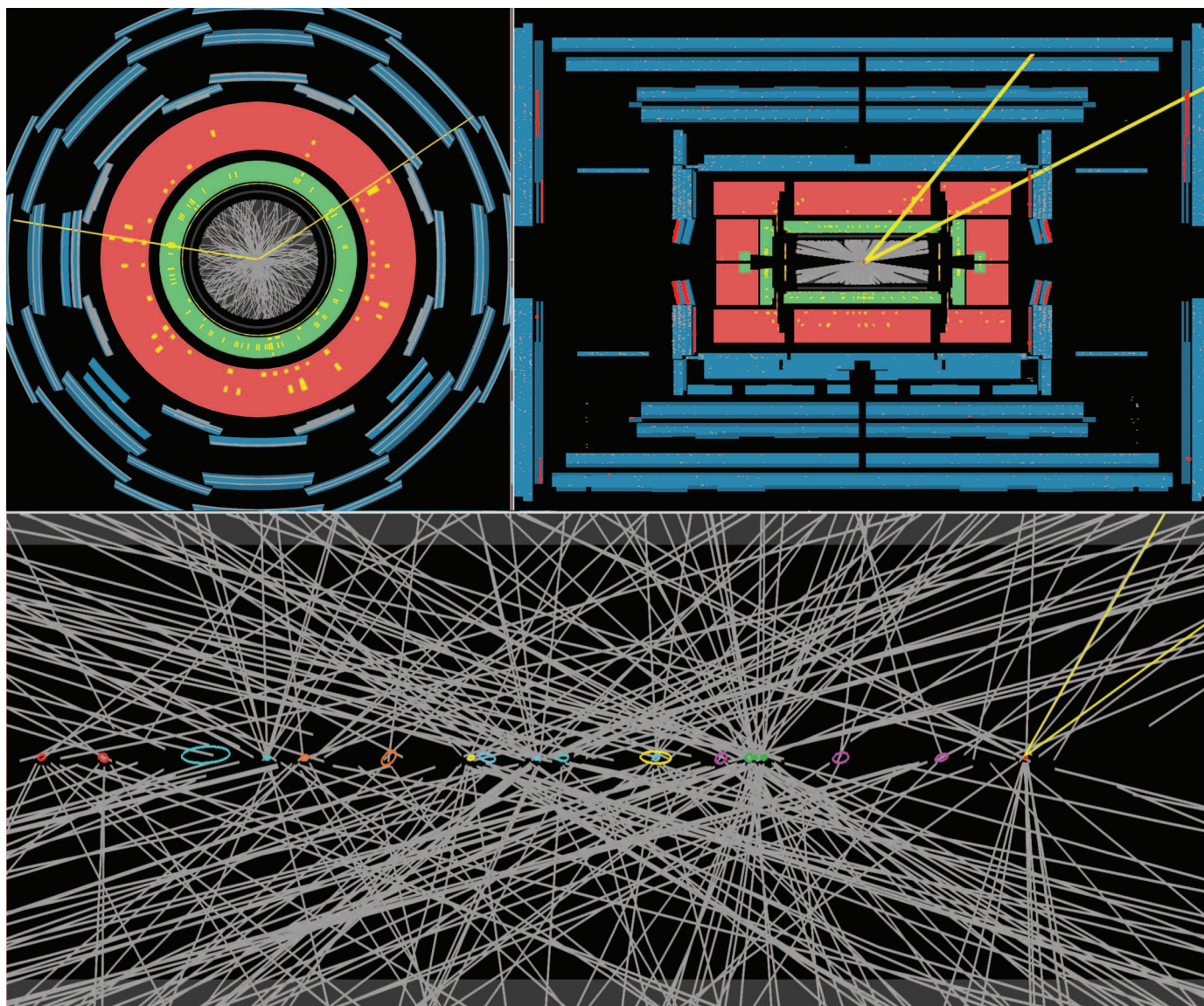


Fig. 2. A candidate Z boson decay to $\mu^+\mu^-$ with 20 reconstructed vertices (the typical pile-up condition in the 8 TeV data). **(Top)** The transverse (left) and longitudinal (right) projections in the full ATLAS detector where the two muons (yellow) are clearly identified. **(Bottom)** The detail of the 20-cm-long vertex region. The two muons can both be seen to emerge from the same vertex. The error ellipses of the reconstructed vertices are shown scaled up by a factor of 10 so that they are visible.

detector is distinctive. Owing to the excellent mass measurement, this channel should show a narrow peak with width $\sim 1.3\%$ in an otherwise featureless $\gamma\gamma$ mass spectrum of background in the search range between 110 and 150 GeV.

The signal-to-background ratio was improved with strict photon identification and other requirements; this reduced the enormous jet background by a factor of $\sim 10^8$ while keeping almost half of the predicted $H \rightarrow \gamma\gamma$ signal events. The majority of the remaining background consists of genuine photon pairs from processes that do not involve a SM Higgs boson. A typical candidate for a $H \rightarrow \gamma\gamma$ decay is shown in Fig. 5. Nearly all such events are from background processes.

The di-photon mass was calculated from the measured properties of the photons. The depth

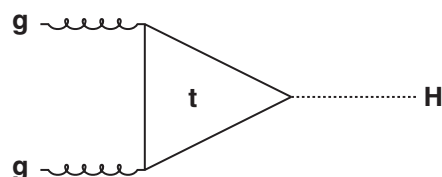


Fig. 3. Feynman diagram illustrating the dominant Higgs boson production mechanism at the elementary level at the LHC: production of a Higgs boson, H, by gluon fusion and a quantum loop process involving a t quark.

segmentation of the calorimeter allows the directions of the photons to be measured. Extrapolating these back to the beam-line gives the position of the production position to an accuracy of 15 mm,

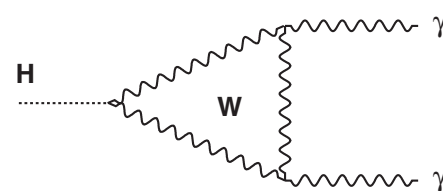


Fig. 4. Feynman diagram illustrating the decay mechanism of a Higgs boson, H, to two photons, γ , via quantum loop process involving a W boson.

which is sufficient to precisely determine the mass. None of the background types forms a sharp peak at any di-photon mass in the search region.

The predicted signal yield in the full set of 59,039 selected events was ~ 190 events for $m_H =$

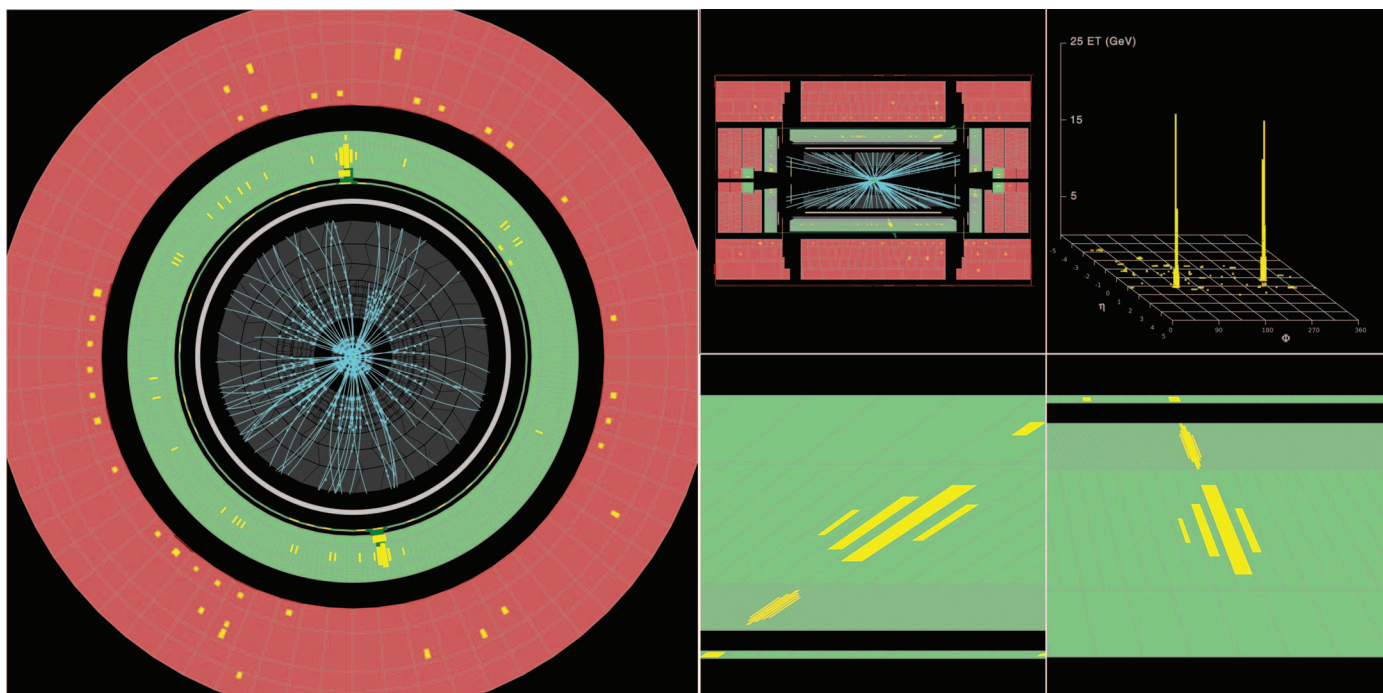


Fig. 5. Display of a $H \rightarrow \gamma\gamma$ event candidate in the 8 TeV data. Energy deposits are shown in yellow in the ECAL (green) and hadronic calorimeter (red). Tracks from charged particles and the associated space points measured by the ID are shown in blue. Views of the calorimeter systems and ID are shown along the proton-proton collision axis (top middle) and transverse to it (left). The bottom middle and bottom right panels show a magnified

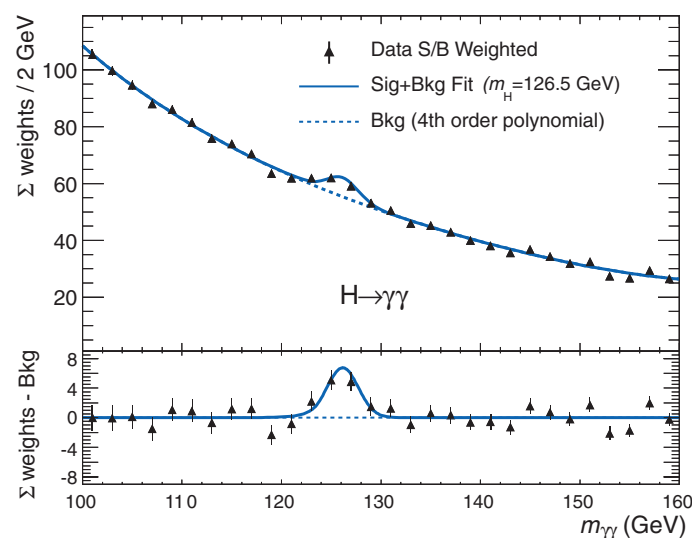
display of the response of the fine-grained ECAL in the longitudinal view for the two photon candidates. Photons are rapidly stopped in the dense ECAL, and these truncated showers match that expectation. The plot on the top right shows the energy depositions projected into azimuthal and longitudinal coordinates—unrolling the calorimeter. The measured mass of this photon-candidate pair is 126.9 GeV.

126 GeV. We enhanced the sensitivity of the analysis by assigning the events to 10 mutually exclusive categories, each with different signal purities and mass resolutions. The signal was extracted by a global fit to the $\gamma\gamma$ mass spectra in the categories. Each spectrum is described by a smooth parametric background model plus a signal model, taken from simulation, which is approximately Gaussian but includes broader tails. For representational purposes, these 10 categories are combined into a single di-photon mass plot, Fig. 6, in which a small but statistically significant excess can be seen.

$H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$ channel. The search channel in which the SM Higgs boson decays to two Z bosons, each then decaying to either e^+e^- or $\mu^+\mu^-$, offers the best signal purity, over 50%. However, because only 7% of Z bosons decay like this, the rate is low: About six signal events were expected in our data sample for $m_H \approx 126$ GeV. Owing to the precise momentum and energy measurements of leptons, a SM Higgs boson with $m_H \approx 126$ GeV will produce a narrow peak of width $\sim 1.5\%$ in the measured four-lepton mass spectrum. The observed four-lepton mass spectrum is consistent with the predicted background, as seen in Fig. 7, apart from the narrow excess of events with mass of ~ 126 GeV.

$H \rightarrow WW \rightarrow \ell\nu\ell\nu$ channel. The decay $H \rightarrow WW \rightarrow \ell\nu\ell\nu$ also provides good sensitivity for $m_H =$

Fig. 6. Distribution of the mass, $m_{\gamma\gamma}$, of weighted di-photon candidates. The selected events are weighted by factors that reflect the signal-to-background ratio predicted for a SM Higgs boson. The result of a fit to the data of the sum of a signal component fixed to $m_H = 126.5$ GeV and a background component described by a fourth-order polynomial are superimposed. The residuals of the weighted data with respect to the fitted background are displayed at the bottom. Collision energy = 7 TeV, integrated luminosity (L) = 4.8 inverse femtobarns (fb^{-1}); collision energy = 8 TeV, $L = 5.9 \text{ fb}^{-1}$.



126 GeV, owing to the relatively large predicted number of events combined with the purely leptonic signature. However, the presence of two undetectable neutrinos in the final state means that a full reconstruction of each event is impossible. Consequently, the masses of the Higgs boson candidates cannot be calculated. However, a “transverse mass,” m_T , which is sensitive to the

Higgs boson mass, was constructed from the detected leptons and missing transverse momentum (31). Because the presence of a signal is primarily inferred via a difference between the event rate observed and that expected from background only, and the expected signal yield is only about 15 to 20% of the background rate in the region of interest, the background rate and composition

The Higgs Boson

must be understood to a precision considerably better than 20%. To accomplish this, we isolated all of the largest background components in kinematically nearby regions of data in which no Higgs boson signal is expected and extrapolated the measured background rates into the signal region. The largest background in the final selection was in fact from direct WW production, which does not involve a Higgs boson.

The E_T^{miss} resolution in the 8 TeV data is degraded by increased pile-up, which increases the background rates, especially for high-mass pairs of leptons of the same type. To circumvent this, we decided to use only events with an electron-muon

pair for the 8 TeV data (one such event is shown in Fig. 8). The distribution of transverse mass for these events is shown in Fig. 9, in which ~ 40 signal events are predicted for $m_H = 126$ GeV.

Statistical procedures. Because the SM makes a specific prediction for how the SM Higgs boson is produced and decays, the results in all production and decay channels and data sets are combined into a single likelihood function (20). The likelihood depends on a signal strength parameter, μ , which is a scale factor on the total number of events predicted by the SM for a Higgs boson signal. It is defined so that $\mu = 0$ corresponds to the background-only hypothe-

sis, and $\mu = 1$ corresponds to the predicted Higgs boson signal in addition to the background.

The likelihood is calculated as the product of the probabilities of observing each event, where the individual event probabilities depend on the measured masses (or m_T) of the Higgs boson candidates. The evaluation accounts for systematic uncertainties. The signal strength and the parameters that describe the systematic uncertainties are varied to maximize the likelihood of the model used to describe the observed data. The ratio of the likelihood with the best-fit signal to that with a specified signal, $\mu = 1$ or 0, is calculated; these likelihood ratios are then used to quantify the exclusion of the signal hypothesis or the rejection of the background hypothesis, respectively.

The statistical tests were repeated at various values of m_H and μ . A SM Higgs boson with mass m_H was considered excluded when $\mu = 1$ is excluded at 95% CL at that mass. This is equivalent to the upper limit on μ at 95% CL being less than 1. On the other hand, a significant rejection of the background hypothesis was interpreted as evidence for the SM Higgs boson because this is the alternate hypothesis. The significance is quantified with the local P value, the probability that the background can randomly fluctuate to produce a measured likelihood ratio at least as signal-like as the excess observed in the data; it is also expressed in terms of the equivalent number of standard deviations of a normal distribution (σ) and is then referred to as the local significance.

Because the SM does not predict the value of m_H , and because background fluctuations can occur anywhere in the search region of m_H , the

Fig. 7. The distribution of the mass of the selected $H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$ candidate events, $m_{\ell\ell\ell\ell}$. The small peak at 90 GeV corresponds to a single Z boson decaying to four leptons, whereas the broad structure around 200 GeV results from the direct production of Z boson pairs. An excess is seen around 125 GeV; the expected signal from a SM Higgs boson at that mass (light blue) is added for comparison. The hatched area indicates the systematic uncertainty in the background estimation. Collision energy = 7 TeV, $L = 4.8 \text{ fb}^{-1}$; collision energy = 8 TeV, $L = 5.8 \text{ fb}^{-1}$.

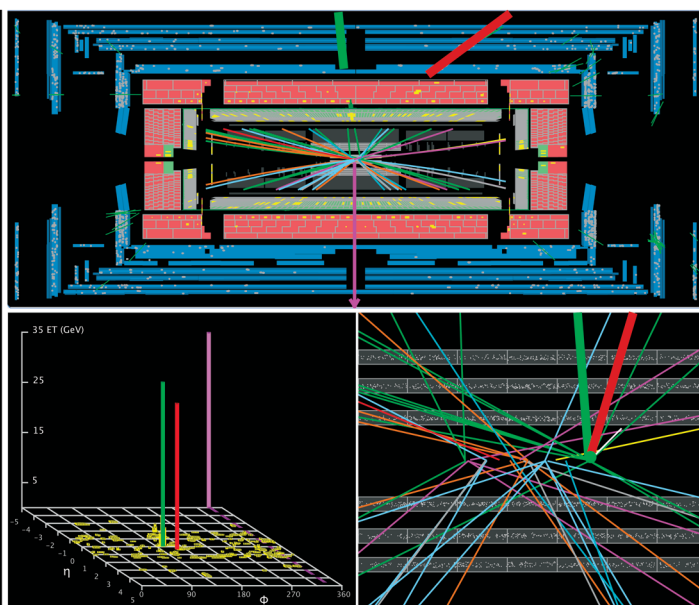
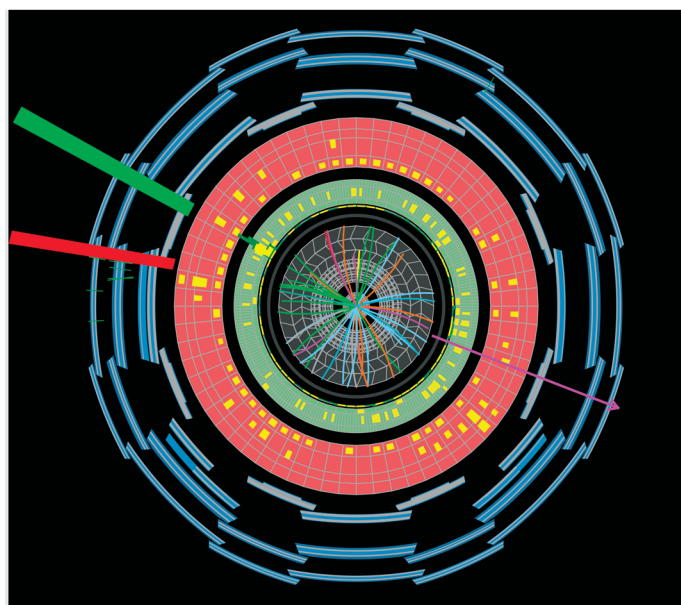
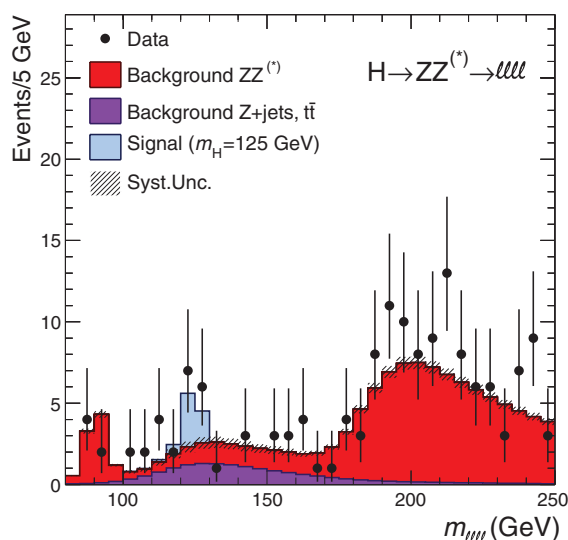


Fig. 8. A candidate event for $H \rightarrow WW \rightarrow e\nu\mu\nu$ shown in transverse (left) and longitudinal (top right) projections through the complete detector. Under the SM Higgs boson hypothesis with $m_H = 126$ GeV, 90% of such events are background. The electron and muon directions are highlighted with green and red markers, respectively. They are on the opposite side of the detector from a

large amount of E_T^{miss} (magenta), and they both come from the same vertex (bottom right). The plot on the bottom middle shows the energy deposition projected into azimuthal and longitudinal coordinates; because E_T^{miss} is only defined in azimuth, the line representing it is arbitrarily placed at the farthest longitudinal position.

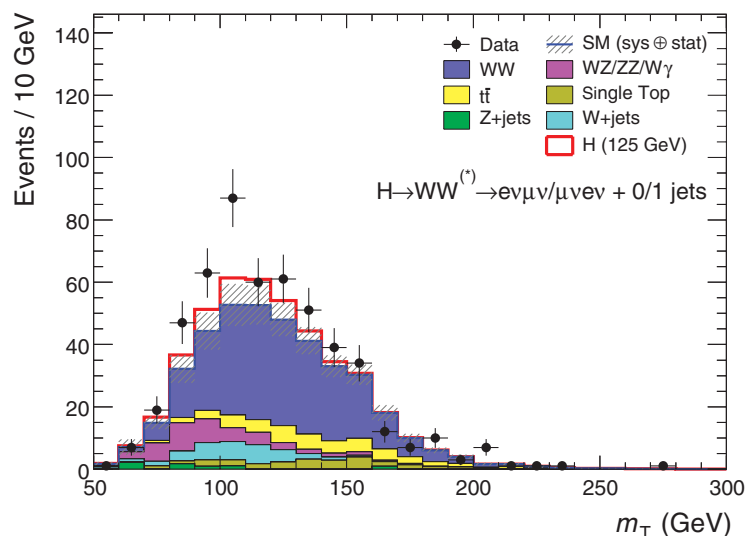


Fig. 9. The distribution of transverse mass, m_T , for $H \rightarrow WW \rightarrow e\nu\mu\nu$ candidates in the 8 TeV data taken during April–June 2012. The red histogram indicates the predicted enhancement of the distribution for $m_H = 125$ GeV. The hatched band indicates the total uncertainty on the background estimation. Collision energy = 8 TeV, $L = 5.8 \text{ fb}^{-1}$.

Fig. 10. Combined search results: the measured (solid) 95% CL upper limits on the signal strength as a function of m_H and the expectation (dashed) under the background-only hypothesis. The green and yellow bands show the $\pm 1\sigma$ and $\pm 2\sigma$ uncertainties on the background-only expectation, respectively. In the broad region where the expected limit is below the signal strength for the SM, $\mu = 1$, there is sensitivity to exclude a Higgs boson in its absence. There are two regions of Higgs boson mass that are not excluded by the data. There is a mild failure to exclude at high mass and a significant failure to exclude around $m_H = 126$ GeV. Collision energy = 7 TeV, $L = 4.6$ to 4.8 fb^{-1} ; collision energy = 8 TeV, $L = 5.8$ to 5.9 fb^{-1} .

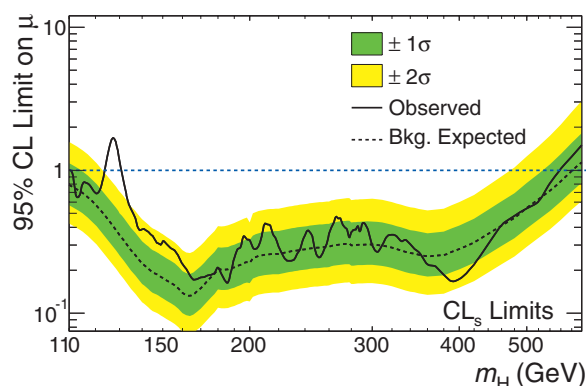
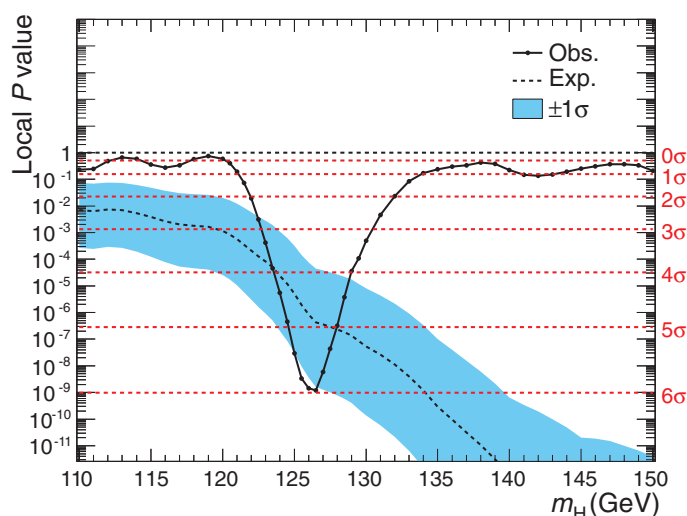


Fig. 11. Combined search results: the observed (solid) local P as a function of m_H and the expectation (dashed) for a SM Higgs boson signal hypothesis ($\mu = 1$) at the given mass. The shaded band indicates the expected $\pm 1\sigma$ variation of the signal prediction. Collision energy = 7 TeV, $L = 4.6$ to 4.8 fb^{-1} ; collision energy = 8 TeV, $L = 5.8$ to 5.9 fb^{-1} .



local significance is an overestimate of the true significance. The global significance corrects for this “look-elsewhere” effect.

Observation of a SM Higgs-like boson. The combined search used the full 7 TeV data collected in 2011 and the 8 TeV data from April–June 2012 in the most sensitive channels. The latter data provide considerable gains in sensitivity with respect to the search based on 2011 data only (20). In the absence of a signal, this should allow the exclusion of the SM Higgs boson for all masses between 110 and 582 GeV, as shown in Fig. 10. This range overlaps with the lower bound from LEP (114.4 GeV); if the entire range had been excluded, this would have shown the SM to be deeply flawed. Our data exclude a SM Higgs boson signal at 95% CL in two mass regions, 111 to 122 GeV and 131 to 559 GeV (Fig. 10). In the region around 126 GeV, this analysis is more than sensitive enough to exclude a SM Higgs boson signal at 95% CL; the failure to do so means that the possibility of a discovery must be considered. Indeed, as shown in Figs. 6, 7, and 9, an excess of events is observed near $m_H = 126$ GeV.

The largest local significance for the combined data is for a SM Higgs boson mass of $m_H \sim 126$ GeV, at which it reaches 6.0σ , corresponding to a probability of an upward fluctuation of the background of 1.0×10^{-9} . This significance is slightly higher than, but consistent with, the expected SM Higgs boson signal at this mass, as seen in Fig. 11. The observed significances for the statistically independent 7 and 8 TeV data samples both peak at ~ 126 GeV, at which they are 3.6σ and 4.9σ , respectively. Uncertainties in the relative energy scales of the detector for electrons and muons reduce the combined local significance to 5.9σ . The global significance of the excess is $\sim 5.1\sigma$.

It is now crucial to establish how well this observation corresponds (or not) to the SM Higgs boson. The consistency of the production rates in the three primary channels with the predictions of the theory is confirmed with a simultaneous fit to μ and m_H , as shown in Fig. 12, in which the CL contours take into account all systematic uncertainties, including the effects of the energy scale and resolution. The positions of the mass peaks in the two channels with the best mass resolution, $H \rightarrow \gamma\gamma$ and $H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$, are consistent with the observation of a single new particle. The measured mass of the observed particle is $126.0 \pm 0.4 \pm 0.4$ GeV, where the two uncertainties are statistical and systematic, respectively. The leading sources of systematic uncertainty come from the photon and, to a lesser extent, electron energy scales.

The signal strength for the fitted mass is $\mu = 1.4 \pm 0.3$, which is consistent with the SM Higgs boson hypothesis $\mu = 1$. Overall, the results in all channels (Fig. 13) are consistent with the SM Higgs boson hypothesis.

Conclusions and outlook. The high degree of statistical significance and simultaneous observation in multiple channels and data sets in this search

The Higgs Boson

Fig. 12. Confidence intervals comparing mass and signal strength for the $H \rightarrow \gamma\gamma$, $H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$, and $H \rightarrow WW \rightarrow \ell\nu\ell\nu$ channels, including all systematic uncertainties. The markers indicate the maximum likelihood estimates (μ , m_H) in the corresponding channels (the maximum likelihood estimates for $H \rightarrow ZZ \rightarrow \ell\ell\ell\ell$ and $H \rightarrow WW \rightarrow \ell\nu\ell\nu$ coincide). Collision energy = 7 TeV, $L = 4.7$ to 4.8 fb^{-1} ; collision energy = 8 TeV, $L = 5.8$ to 5.9 fb^{-1} .

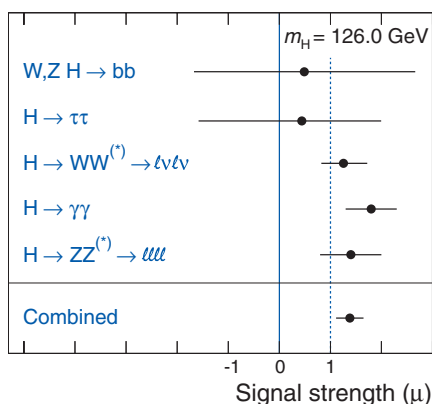
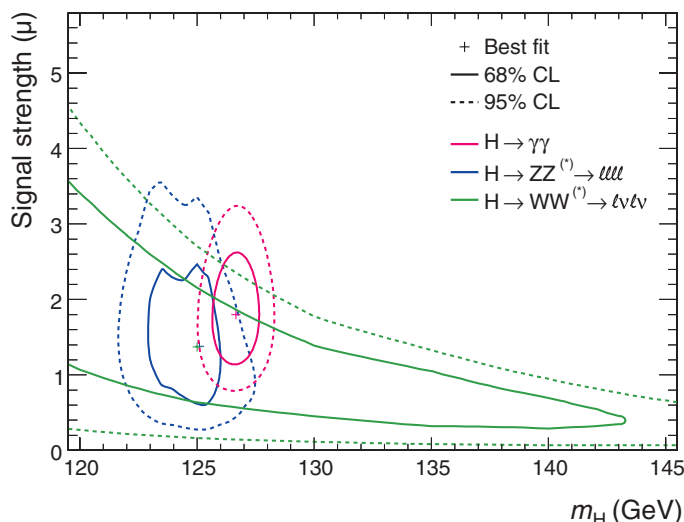


Fig. 13. Measurements of the signal strength parameter μ for $m_H = 126 \text{ GeV}$ for the individual channels and their combination. The vertical dotted line at $\mu = 1$ indicates the expectation for a SM Higgs boson. Collision energy = 7 TeV, $L = 4.6$ to 4.8 fb^{-1} ; collision energy = 8 TeV, $L = 5.8$ to 5.9 fb^{-1} .

for the SM Higgs boson demonstrate that we have observed a new particle with properties consistent with those of the SM Higgs boson. The CMS experiment has independently reported results (23, 24) in striking agreement with ours. In addition, evidence for a boson at the level of three standard deviations in the mass region of 120 to 135 GeV has been reported recently by experiments at the Tevatron proton-antiproton collider at Fermilab (32).

The observation of the new particle in the $\gamma\gamma$, ZZ , and WW decay modes shows that it is an electrically neutral boson and supports the extraordinary prediction of the SM that the vacuum is not void, but filled with a field of weak charge. The spin of the new particle, expected to be 0 if it is a Higgs boson, is not yet determined, but spin-1 particles cannot decay into two photons (33, 34). It is also important to test whether the rates of its decays to the quarks and leptons match the predictions for the SM Higgs boson. Our searches

for decays to b quarks and τ leptons are not yet sensitive enough to give conclusive results.

The energy density of the SM vacuum field is predicted to fill all of space. The nonzero vacuum energy density can be verified further by measuring the Higgs boson self-coupling, which can be accomplished by observing the production of multiple Higgs bosons. Because the expected production rate is small, this will be a challenge for the future high-intensity LHC program. General relativity normally associates a gravitational attraction to energy density. The impact of the energy density of the SM vacuum field on cosmology should be large. Because such effects are not observed, the relationship between the SM vacuum energy density and gravitation remains to be explored.

A relatively light Higgs boson suggests that new physical phenomena may exist at energies not far above the measured mass. Without new phenomena, quantum loop processes would drive the predicted Higgs boson mass far above the highest energy scale at which the SM is valid. For example, a theoretical model known as supersymmetry could provide a natural explanation for the light mass. Supersymmetry unifies matter and forces and for every known particle predicts a new “superpartner,” some of which would enter into the quantum loops affecting the Higgs boson mass. The ATLAS and CMS experiments are actively searching for the first direct evidence of these superpartners. Various models, including supersymmetry, suggest that five distinct types of Higgs bosons exist. Therefore, another key issue is whether the observed boson is the only Higgs boson.

The groundbreaking discovery of a SM Higgs-like boson may have identified the last missing piece of the SM as originally envisaged but is also an inspiration for further studies of the newly discovered boson, which might be a means to explore the physics that must lie beyond the SM. The LHC and its experiments are expected to address this new challenge in the coming years.

References and Notes

1. S. L. Glashow, *Nucl. Phys.* **22**, 579 (1961).
2. S. Weinberg, *Phys. Rev. Lett.* **19**, 1264 (1967).
3. A. Salam, in *Elementary Particle Theory*, N. Svartholm, Ed. (Almqvist and Wiksell, Stockholm, 1968), pp. 367–377.
4. G. 't Hooft, M. Veltman, *Nucl. Phys. B* **44**, 189 (1972).
5. F. Englert, R. Brout, *Phys. Rev. Lett.* **13**, 321 (1964).
6. P. W. Higgs, *Phys. Lett.* **12**, 132 (1964).
7. P. W. Higgs, *Phys. Rev. Lett.* **13**, 508 (1964).
8. G. S. Guralnik, C. R. Hagen, T. W. B. Kibble, *Phys. Rev. Lett.* **13**, 585 (1964).
9. P. W. Higgs, *Phys. Rev.* **145**, 1156 (1966).
10. T. W. B. Kibble, *Phys. Rev.* **155**, 1554 (1967).
11. ALEPH, CDF, DØ, DELPHI, L3, OPAL, SLD Collaborations, LEP Electroweak Working Group, Tevatron Electroweak Working Group, SLD electroweak and heavy flavour groups, <http://arxiv.org/abs/1012.2367> (2010).
12. More recent results are available at <http://lepewwg.web.cern.ch/LEPEWWG/plots/winter2012>.
13. ALEPH, DELPHI, L3, OPAL, SLD Collaborations, LEP Electroweak Working Group, SLD Electroweak and Heavy Flavour Groups, *Phys. Rep.* **427**, 256 (2006).
14. In the literature of particle physics, it is common to use natural units where the value of the velocity of light in vacuum is 1, so that mass, momentum, and energy all have units of electron volts.
15. LEP Working Group for Higgs boson searches, ALEPH, DELPHI, L3, OPAL, *Phys. Lett. B* **565**, 61 (2003).
16. CDF Collaboration, DØ Collaboration, Tevatron New Physics, Higgs Working Group, <http://arxiv.org/abs/1207.0449> (2012).
17. L. Evans, P. Bryant, Eds., *JINST* **3**, S08001 (2008).
18. ATLAS Collaboration, *JINST* **3**, S08003 (2008).
19. CMS Collaboration, *JINST* **3**, S08004 (2008).
20. ATLAS Collaboration, *Phys. Rev. D* **86**, 032003 (2012).
21. CMS Collaboration, *Phys. Lett. B* **710**, 26 (2012).
22. ATLAS Collaboration, *Phys. Lett. B* **716**, 1 (2012).
23. CMS Collaboration, *Phys. Lett. B* **716**, 30 (2012).
24. CMS Collaboration, *Science* **338**, 1569 (2012).
25. M. Della Negra, P. Jenni, T. S. Virdee, *Science* **338**, 1560 (2012).
26. LHC Higgs Cross Section Working Group, S. Dittmaier, C. Mariotti, G. Passarino, R. Tanaka, Eds., <http://arxiv.org/abs/1101.0593> (2011).
27. LHC Higgs Cross Section Working Group, S. Dittmaier, C. Mariotti, G. Passarino, R. Tanaka, Eds., <http://arxiv.org/abs/1201.3084> (2012).
28. ATLAS Collaboration, *Eur. Phys. J. C* **70**, 823 (2010).
29. S. Agostinelli et al., *Nucl. Instrum. Methods A* **506**, 250 (2003).
30. The symbol ℓ stands for electron, e , or muon, μ , for the decay modes examined in this article.
31. A. J. Barr, B. Gripaios, C. G. Lester, *J. High Ener. Phys.* **0907**, 072 (2009).
32. T. Aaltonen et al., CDF Collaboration, DØ Collaboration, *Phys. Rev. Lett.* **109**, 071804 (2012).
33. L. D. Landau, *Dokl. Akad. Nauk. USSR* **60**, 207 (1948).
34. C. N. Yang, *Phys. Rev.* **77**, 242 (1950).

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Supplementary Materials

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Radar-Enabled Recovery of the Sutter's Mill Meteorite, a Carbonaceous Chondrite Regolith Breccia

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Doppler weather radar imaging enabled the rapid recovery of the Sutter's Mill meteorite after a rare 4-kiloton of TNT-equivalent asteroid impact over the foothills of the Sierra Nevada in northern California. The recovered meteorites survived a record high-speed entry of 28.6 kilometers per second from an orbit close to that of Jupiter-family comets (Tisserand's parameter = 2.8 ± 0.3). Sutter's Mill is a regolith breccia composed of CM (Mighei)-type carbonaceous chondrite and highly reduced xenolithic materials. It exhibits considerable diversity of mineralogy, petrography, and isotope and organic chemistry, resulting from a complex formation history of the parent body surface. That diversity is quickly masked by alteration once in the terrestrial environment but will need to be considered when samples returned by missions to C-class asteroids are interpreted.

On 22 April 2012, the KBBX (Beale Air Force Base, California), KDAX (Sacramento, California), and KRGX (Reno, Nevada) weather radars of the U.S. National Climatic Data Center's NEXRAD network (*1*) detected radial Doppler shifts in four sweeps, following a fast-moving daytime fireball seen over much of California and Nevada at 14:51:12 to 17 UTC (Fig. 1). The falling meteorites were

identified from a downward sequence of subsequent detections, small-scale turbulence, and widely variable spectrum width values correlated in time, location, and direction with eyewitness reports of the fireball.

Under the radar footprint over the townships of Coloma and Lotus in El Dorado County, California, the first three pieces of the meteorite were recovered on 24 April, before heavy rain hit

the area (*2*). One meteorite fell at Sutter's Mill (SM), the gold discovery site that initiated the California Gold Rush. Two months after the fall, SM find numbers were assigned to the 77 meteorites listed in table S3 (*3*), with a total mass of 943 g. The biggest meteorite is 205 g.

This is a tiny fraction of the pre-atmospheric mass, based on the kinetic energy derived from infrasound records. Eyewitnesses reported hearing a loud boom followed by a deep rumble. Infrasound signals (table S2A) at stations I57US and I56US of the International Monitoring System (*4*), located ~770 and ~1080 km from the source, are consistent with stratospherically ducted arrivals (*5*). The combined average periods of all phase-aligned stacked waveforms at each station of 7.6 s correspond to a mean source energy of 4.0 (–2.2/+3.4) kT of TNT, using the multistation period yield relation from (*5*). This was the most energetic reported bolide falling on land globally (*6*) since the 1.2-kT impact of asteroid 2008 TC₃ over Sudan in 2008 (*7*).

Seismic data suggest a point source altitude of 54.8 ± 10.9 km above mean sea level, estimated from impulsive phase arrivals of the air blast at eight seismograph stations (*8*) by applying a simple half-space sonic velocity model and direct ray paths to a standard earthquake location code (table S2B).

This altitude corresponds to a persistent flare detected in a set of three photographs from Rancho Haven, north of Reno, Nevada (fig. S1). Triangulation with videos from Johnsondale, California, and Incline Village, Nevada (figs. S2 and S3), shows that the bolide was first detected at 90 km approaching from the east, had a broad peak in brightness around 56 km, and detonated at 47.6 ± 0.7 km (Table 1). Even in the daytime sky, a great many fragments were detected down to 30 km.

The entry speed is twice that of typical triangulated falls from which meteorites have been recovered (table S1). SM has the highest disruption altitude on record. With an entry velocity of 28.6 km/s, the infrasound-derived kinetic energy corresponds to a pre-atmospheric mass of ~40,000

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(range 20,000 to 80,000) kg. Counter to intuition, the catastrophic disruption was key to meteorite survival from this fast entry (7). So far, ~0.1 kg/km has been recovered along the trend line, for an estimated total fallen mass ≥1.7 kg. This is far less than that recovered from the similar-sized but slower-impacting 2008 TC₃: 1.2 kg/km and 39 kg (7). An airship search did not find impact scars from falling kilogram-sized meteorites.

The pre-atmospheric orbit (Table 1) had low inclination and high eccentricity, with aphelion close to the orbit of Jupiter and perihelion (*q*) approaching the orbit of Mercury (fig. S5). Semimajor axis, eccentricity, and inclination are strikingly similar to the preliminary values reported for the CM-type carbonaceous chondrite Maribo (9) by Haack *et al.* (10). The entry conditions, too, were much alike. Upon request, W. Singer and G. Stober provided the seven Juliusruh radar head-echo range and direction positions of the early 112- to 79-km altitude part of this fall (table S1), which translated into the orbital elements listed in Table 1. SM and Maribo have lower perihelion distance and higher-eccentricity orbits than all other known (mostly ordinary chondrite) falls (table S1).

That distinction may be due to a bias toward a more recent evolution into Earth-crossing orbits for this population. SM has a cosmic ray exposure (CRE) age at the extreme low end of the CM2 chon-

drite CRE age distribution, which as a group is younger than all other classes of meteorites except lunar meteorites (11). An age of 0.10 ± 0.04 million years (My) was obtained from the measured ²⁶Al activity (with a 0.705 million-year half-life) of 3.8 ± 0.8 dpm/kg in SM36 (table S20). He and Ar isotopic ratios in SM43 and SM51 show no clear excess of a cosmogenic noble gas component, but a small excess of cosmogenic Ne is observed as deviations from two mixing lines between the trapped components; solar Ne – P3 Ne and P3 Ne – Ne E (12, 13) (fig. S27). The low concentration of cosmogenic ²¹Ne [1.02 ± 0.11 × 10⁻¹⁰ cm³/g at standard temperature and pressure (STP)] indicates a very short CRE age of 0.051 ± 0.006 My (the average of four ages in table S18), if a production rate of 2 × 10⁻⁹ cm³/g/My at STP for the near surface of a large object is adopted (14).

The SM and Maribo orbits have a Tisserand’s parameter with respect to Jupiter (*T_J*) that borders those of asteroids (*T_J* > 3) and Jupiter family comets (*T_J* = 2 to 3). Visual observations of Murchison (CM) also point to approach on a low-inclined orbit (15). A possible Jupiter-family comet origin is intriguing (10) [for a review, see (15)], especially because the CM-like micrometeorites are dynamically linked to Jupiter-family comets (16). Finding two asteroids on orbits at *q* = 0.47 astronomical unit (AU) could mean that both are part

of an old ~0.1-My-old meteoroid stream, perhaps related to 2P/Encke (10), but only if CM chondrites survive longer than typical Taurid meteoroids.

Until other evidence of hydrothermal alteration in Jupiter-family comets is found (15), an origin in the asteroid belt is more likely. The asteroid-family source has low inclination and is close to the 3:1 mean motion resonance with Jupiter, the CRE age leaving little time for thermal drag forces to move the semimajor axis of a main-belt asteroid into resonance at 2.5 AU. A suitable candidate is the (495) Eulalia family, recently proposed as a source of near-Earth C-class asteroids (17). Unlike ordinary chondrites ejected from the 3:1 resonance, which tend to collide with Earth on higher-impact-probability perihelion distance *q* ~ 1 AU orbits of lower eccentricity (table S1), the fractured CM chondrites may disintegrate too rapidly to evolve into such orbits. The short exposure age of SM as compared to other CM chondrites could mean it had already broken from a larger precursor while evolving into an SM-like orbit.

The reflectance spectrum of SM12 (fig. S8) is a good match to the Hayabusa 2 mission’s target, asteroid 1999 JU₃, over the measured 0.38- to 0.92-μm range when normalized at 0.55 μm (fig. S8). The meteorite’s albedo at 0.55 μm is low, 2.5 to 4.0% at the standard 30° incidence and 0° emergence angles. Carbonates are abundant in this sample and produce strong infrared absorption bands near 1450 and 875 cm⁻¹, stronger than those seen in CM2 Murchison.

SM12 has a compression strength of 82 ± 6 MPa, as compared to ~30 MPa for other CM2s (18). The impacting asteroid fragmented at a dynamic pressure of only 0.9 MPa at 48 km, presumably because of internal cracks (19). X-ray computed tomography scanning (20) of SM3, 9, 18, 51, 54, and 73 at the 12- to 30-μm/voxel edge showed abundant fractures through stones and abundant fractures within some lithic fragments, terminating at the fragment edges (fig. S28). Volume measurements (table S19) yielded densities of 2.27 ± 0.07 g/cm³, similar to an average CM2 density of 2.20 g/cm³ (range 1.88 to 2.47) (21). He ideal-gas pycnometry of SM19 gave a bulk density of 2.31 ± 0.04 g/cm³, a grain density of 3.34 ± 0.02 g/cm³, and a high porosity of 31.0 ± 1.4% (22).

This density and the infrasound-derived kinetic energy yield an asteroid diameter of 2.5 to

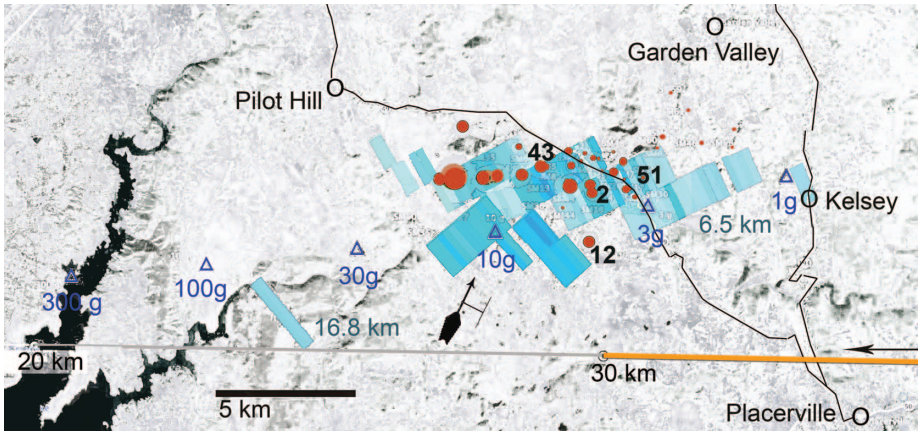


Fig. 1. Map of ground-projected fireball trajectory solution (orange line), radar Doppler reflectivity (light blue; -5 dBZ for pale blue to +15 dBZ for deepest blue), and meteorite find locations near Sutter’s Mill (red dots are of size proportional to mass; relevant finds are labeled with SM number). Blue triangles are the calculated impact locations for breakup at 48 km and wind drift from 13 m/s winds to azimuth 27° between 18 and 2 km altitude.

Table 1. Atmospheric trajectory and pre-atmospheric orbit for the SM and Maribo CM chondrites. Angular elements are for equinox J2000.0.

Atmospheric trajectory	SM	Maribo	Pre-atmospheric orbit	SM	Maribo
<i>H_b</i> (beginning height - km)	90.2 ± 0.4	111.8 ± 0.4	<i>T_J</i> (Tisserand’s parameter)	2.81 ± 0.32	3.04 ± 0.32
<i>H_m</i> (broad maximum - km)	~56	~58	<i>a</i> (semimajor axis - AU)	2.59 ± 0.35	2.34 ± 0.29
<i>H_f</i> (disruption - km)	47.6 ± 0.7	37.3 ± 0.6	<i>e</i> (eccentricity)	0.824 ± 0.020	0.795 ± 0.026
<i>H_e</i> (end height - km)	~30.1	~32	<i>q</i> (perihelion distance - AU)	0.456 ± 0.022	0.481 ± 0.010
<i>V_∞</i> (entry speed - km/s)	28.6 ± 0.6	28.0 ± 0.7	<i>ω</i> (argument of perihelion - °)	77.8 ± 3.2	99.0 ± 1.4
<i>h</i> (entry elevation angle - °)	26.3 ± 0.5	30.2 ± 0.5	<i>Ω</i> (longitude of ascending node - °)	32.77 ± 0.06	117.64 ± 0.05
<i>a_z</i> (entry azimuth angle from south - °)	272.5 ± 0.4	276.2 ± 0.2	<i>i</i> (inclination - °)	2.38 ± 1.16	0.72 ± 0.98
<i>V_g</i> (geocentric entry speed - km/s)	26.0 ± 0.7	25.4 ± 0.8	<i>Q</i> (aphelion distance - AU)	4.7 ± 0.7	4.2 ± 0.6
<i>Ra_g</i> (geocentric right ascension of radiant - °)	24.0 ± 1.3	124.6 ± 1.0	<i>T_p</i> (perihelion time)	2012-03-09.1	2008-12-03.6
<i>Dec_g</i> (geocentric declination of radiant - °)	12.7 ± 1.7	18.8 ± 1.6	Epoch (UT)	2012-04-22.620	2009-01-17.798

4.0 m. Concentrations of the cosmogenic radionuclide ^{60}Co in three fragments—SM18, SM36 (table S20A), and SM43—and model calculations (23) confirm that the asteroid's pre-atmospheric size was >0.9 m. The fireball's peak luminosity (-18 to -20 magnitude) at the first broad maximum suggests a size of 1.8 to 3.5 m.

Textural and compositional variety. Because of its large size, SM provides insight into the variety of materials present at the surface of its parent body. Individual SM meteorites have differing magnetic susceptibilities (χ in 10^{-9} m^3/kg). Ten different stones (table S4) suggest a bimodal distribution clustering around $\log_{10}\chi = 4.03$ and 4.26. The primary magnetic mineral is magnetite (Fe_3O_4), with concentrations of 2.0 and 3.3 weight % (wt %), respectively. These magnetite concentrations are intermediate between typical CM2s (~ 1 wt %) and magnetite-rich C2s or anomalous CMs (>6 wt %) (24). SM2 has a stable natural remnant magnetization, probably extraterrestrial in origin, corresponding to a magnetic field paleointensity of ~ 3 μT , comparable to other carbonaceous chondrites (25).

SM is a regolith breccia. Like all CM chondrites (26), SM contains Ne from solar wind implantation in a surface regolith (fig. S27). Unlike most other CM chondrites, the brecciated nature of that regolith is evident: SM2, 18, 47, 48, 51, and 54 contain angular to rounded clasts embedded in a fine-grained comminuted matrix seen visually (Fig. 2A), by x-ray and backscattered electron mapping (Fig. 2B and fig. S13), and by x-ray and neutron computed tomography (figs. S28 and S30).

The classification of SM as a CM chondrite is confirmed by whole-rock chemistry (Fig. 3) and by O (Fig. 4 and fig. S21), Os (supplementary text S2.6), and Cr (supplementary text S2.7) isotopic compositions. The Os isotopic compositions and highly siderophile element abundances are well within the range of CM chondrites (figs. S16 and S17). However, the Re-Os isotopic systematics indicate minor, what was probably recent, open-system behavior of these two elements, as seen in other chondrites (27). SM plots in the field of CM chondrites (28) on the diagram of $\epsilon^{54}\text{Cr}$ versus $\Delta^{17}\text{O}$ (fig. S20). Using ^{53}Mn - ^{53}Cr chronometry (^{53}Mn decays to ^{53}Cr with a half-life of 3.7 My), the data place the accretion time of SM at 4566.57 ± 0.66 million years ago (Ma) (fig. S19) (28).

SM contains CM lithologies (from CM2.0 to 2.1, Fig. 2B) with varying histories of aqueous alteration and thermal metamorphism. The abundances of thermally labile elements, such as Se, Te, Zn, Sn, and Tl (Fig. 3 and table S7), known to be sensitive to open-system heating and volatilization, indicate that most of SM avoided metamorphic (on a million-year time scale) heating above 400° to 500°C (29). Like other CM chondrites, both lithologies of SM51 (Fig. 2B) and some of those in SM2 contain abundant carbonate grains and complete chondrule pseudomorphs embedded in a phyllosilicate-rich matrix (Fig. 2, B and C). Sample SM2-5 contains clasts of incompletely altered CM material, whose matrix consists largely of

submicrometer-sized olivine pseudomorphs after phyllosilicates and troilite (Fig. 2D and fig. S14). This and the complete lack of carbonates and tochilinite indicate that this particular clast has experienced thermal metamorphism to $\sim 500^\circ\text{C}$; all other metamorphosed CMs are finds (30).

Thermoluminescence (TL) measurements (31) of SM2-1d (fig. S35) show heating to $300^\circ \pm 20^\circ\text{C}$ within the last 0.2 My (fig. S36), and it has induced TL similar to low-metamorphic-grade CO and CV chondrites and unlike other CM chondrites. Raman spectra of macromolecular carbon (fig. S33) suggest that SM2-9 experienced only $153^\circ \pm 27^\circ\text{C}$, whereas a sample of SM12 experienced $268^\circ \pm 42^\circ\text{C}$ on a million-year time scale [using the method in (32)]. In a Raman G-band center-versus-width diagram (fig. S33), SM2-9 plots between CM2 and CO3 chondrites, whereas SM12 trends closer to polycrystalline C observed in CV3 chondrites.

There are various potential sources for the observed heating. The fast entry would have heated the meteorite surface to $>700^\circ\text{C}$ for up to 1.5 s, but it is unclear that this would offer enough time to alter the meteorite deeper inside. At 0.47 AU from the Sun, small tumbling asteroids warm up to $\sim 200^\circ\text{C}$, more at the surface, less inside. Alternatively, the recent heating could have been related to the impact that liberated the meteorite from the asteroid surface ≥ 0.05 Ma. Annealing

above 300°C may also have occurred on the parent body during the first 1 My after accretion, the most probable cause being the decay of live ^{26}Al .

SM, like Kaidun, shows large variations in O isotope compositions, reflecting the presence of diverse types of lithologies in both regolith breccias (Fig. 4). The aqueous alteration that produced carbonates and phyllosilicates caused variation in $\delta^{18}\text{O}$ along an approximately fixed $\Delta^{17}\text{O}$ ($=\delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$) fractionation line (Fig. 4). This requires water-rock reaction involving flowing water as a result of a temperature gradient (33). Individual SM fragments plot at different positions along this line. The carbonates exhibit a very large range in $\delta^{18}\text{O}$ value [$+13$ per mil (‰) to $+39$ ‰] with $\Delta^{17}\text{O} = -1.9 \pm 1.5$ ‰ (fig. S21). A trend line regressed through the calcite data, $\delta^{17}\text{O} = -4.88 + 0.62 \times \delta^{18}\text{O}$, is nearly identical to that reported by (34) based on CM calcites.

SM has the lowest N/C ratio and $\delta^{15}\text{N}$, as compared to other CM2 chondrites (fig. S24), suggesting that it contains different N-bearing organic components. C and N isotopic compositions vary widely between fragments, with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranging between -13 ‰ and $+28.5$ ‰ and between -0.6 ‰ and $+16.7$ ‰, respectively. Bimodality in C release suggests two separate organic components, a volatile-rich and a volatile-poor component, the former being isotopically lighter and more cross-linked. Added to these components is CO_2 from

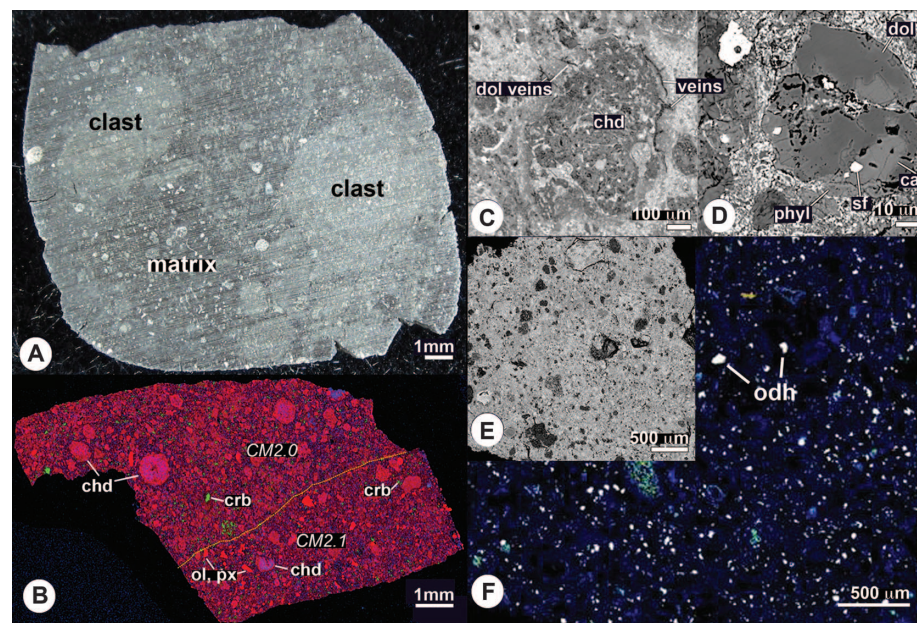


Fig. 2. (A) Slice of SM48 in visible light, showing light clasts in a dark matrix. (B) Combined elemental map in Mg (red), Ca (green), and Al (blue) Ka x-rays of the sample SM51-1 composed of two extensively aqueously altered CM2.0 and CM2.1 lithologies, with a sharp boundary (yellow dashed line). Both lithologies contain complete chondrule pseudomorphs (chd) embedded in a phyllosilicate-rich matrix and abundant carbonate grains (crb). In addition, the CM2.1 lithology contains rare olivine (ol) and pyroxene (px) grains of incompletely hydrated chondrules, amoeboid olivine aggregates (AOAs), and Ca-Al-rich inclusions (CAIs). (C) Backscattered electron (BSE) image of a chondrule pseudomorph (chd) composed mainly of phyllosilicates in SM51-1. The fine-grained rim around the chondrule is crosscut by veins of dolomite (dol), indicating in situ aqueous alteration. (D) BSE image of a carbonate grain composed of closely intergrown dolomite (dol) and calcite (cal) with inclusions of Fe,Ni-sulfide (sf) in SM51-1. The grain is rimmed by phyllosilicates (phyl). (E) BSE image of a small chip from sample SM2-5 with (F) corresponding Ca-S composite x-ray map (Ca, blue; S, green); all light spots in the map correspond to oldhamite (CaS; odh).

the decomposition of carbonates. The maximum in $\delta^{13}\text{C}$ of +65‰ is likely to be from the decomposition of calcite; $\delta^{13}\text{C}$, however, may be underestimated because of contamination by isotopically lighter components. About 2% of the total C combusts above 1000°C and reaches a $\delta^{13}\text{C}$ of +130‰. This is a well-known feature of carbonaceous chondrites, produced by the combustion of ^{13}C -enriched ($\delta^{13}\text{C} \sim +1400$ ‰) presolar silicon carbide grains (35). Apparently, SM contains a significant abundance of presolar grains.

Reactive compounds. The rapid pre-rain recovery of SM offers a rare glimpse of what reactive minerals and organic compounds may be present at the surface of asteroids. Unseen in other CM chondrites, abundant CaS grains were found in SM2-4 (Fig. 2E). A powder Laue pattern of these CaS grains proved to index as oldhamite (fig. S16). Oldhamite is quickly lost to moisture. The oldhamite grains are set within fine-grained comminuted matrix, containing also olivine, enstatite, Fe-Ni-Zn sulfides, Fe-Ni-Cr phosphides, and grains of reduced C, suggesting

admixture of a reduced component, possibly xenolithic enstatite chondrite material. It is interesting that the comminuted matrix of SM contains fragments of such a rare meteorite type, rather than the far more abundant ordinary chondrites, probably implying interactions between C- and E-type asteroids.

In comparison to SM2 (pre-rain), the SM12 sample (post-rain) shows strong alteration effects, demonstrating rapid reaction between terrestrial water and reactive S-bearing species. Fourier transform ion cyclotron resonance mass spectra (FTICR-MS) (36) of SM's methanol soluble fraction showed only hundreds of mass signals with comparatively low intensity, high aliphaticity, and, different from the thousands analyzed in other carbonaceous chondrites, many highly oxygenated species and polysulfur-rich compounds (fig. S31). Nuclear magnetic resonance (NMR) spectroscopy of the same extracts showed abundant highly branched, singly oxygenated aliphatics and a considerable diversity of unsaturated compounds in an intensity ratio of near 40:6:1 (fig. S32). Both NMR and FTICR-MS signals confirmed convergence with structures recently observed in rather highly thermally altered meteorites.

Water-soluble organic compounds and inorganic salts can be formed, mobilized, and altered by aqueous alteration and terrestrial weathering. Ion chromatography (37) of ≤ 20 mg of water-extracted pre-rain SM2 showed formate at 80 parts per million (ppm) and acetate at 700 ppm, whereas post-rain interior SM12 had trace formate and only ~ 100 ppm acetate. Detected inorganic anions in SM2 were sulfate (1300 ppm) and chloride (262 ppm), again with only trace amounts present in interior SM12. Murchison (presumably more aqueously altered) has a 20 times higher soluble sulfate abundance (26,000 ppm) (37) than SM2. Sodium was the dominant cation (on a per-mole basis) at ~ 1900 ppm in SM2, followed by calcium (2080 ppm) and magnesium (117 ppm).

Water extracts of SM2 were analyzed by gas chromatography mass spectrometry for ammonia and amines; amino, hydroxyl, and dicarboxylic acids (38); and, for amino acids, also by liquid chromatography with fluorimetric detection and time-of-flight mass spectrometry (39). The most abundant water-soluble compounds detected by these methods included glycine, β -alanine, γ -amino-*n*-butyric acid, and, in some analyses only, ϵ -amino-*n*-caproic acid (table S21). The highest total amino acid abundances were found in a fragment of SM12 at a depth of 9 to 12 mm from the crust. Exterior portions of SM2 and SM12 contained predominantly contaminant L-amino acids, and both samples were highly depleted in amino acids overall. The pH values of SM water extracts ranged from 8.90 to 9.65, which are higher than for other CMs.

Dichloromethane/methanol (9:1 volume to volume) extracts also showed variably low amounts of soluble hydrocarbon (fig. S34). SM2 contained naphthalene, methylnaphthalenes, dimethylnaphthalenes, anthracene/phenanthrene, a series of linear 15-C to 22-C alkanes, and very little S, usually abundant in comparable CM extracts (40). The SM12 extracts were instead dominated by cyclic

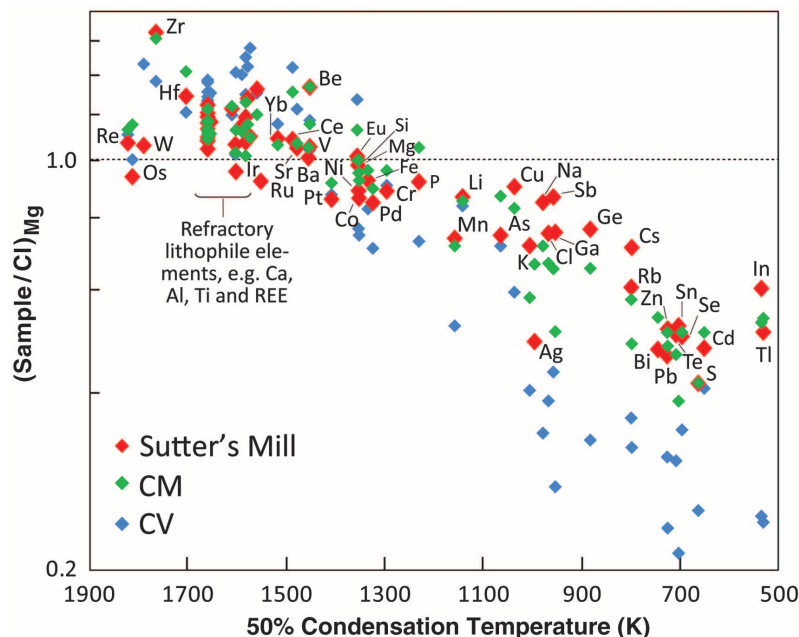


Fig. 3. Average elemental composition of SM meteorite (table S7) compared to averages for CI (Ivuna type), CM, and CV (Vigarano type) groups of carbonaceous chondrites. Data are normalized to CI and Mg and plotted against 50% condensation temperatures of the elements (41). Data sources: CI (41), CV (42), and CM (43).

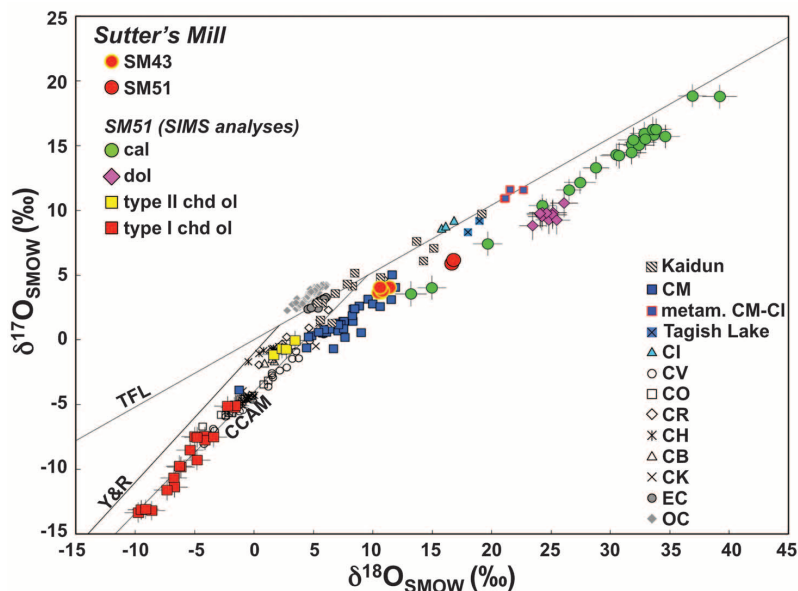


Fig. 4. SM43 and SM51 extend the currently known CM field in the three O-isotope diagram. Data for ordinary, enstatite, and carbonaceous chondrites and for Kaidun chondritic regolith breccia are from (43–47). TFL, terrestrial fractionation line; CCAM, carbonaceous chondrite anhydrous mineral line (44); Y&R, Young and Russell line (48). SMOW, standard mean ocean water.

octatomic S, dimethyltrisulfide, and dimethyl-tetrasulfide, the latter two for about a combined 1 mmole/g; the fragment contained in addition only naphthalene at 2 to 8 nmol/g, plus methyl-naphthalenes and biphenol in subnanomole amounts, but no alkanes or anthracene/phenanthrene.

The SM meteorite demonstrates that the complexity of C-class asteroid surfaces is greater than previously assumed. Rapid terrestrial alteration probably erases many vestiges of the internal and external processes on the asteroid that remain to be explored in spacecraft sample-return missions.

References and Notes

- M. Fries, J. Fries, *Meteorit. Planet. Sci.* **45**, 1476 (2010).
- P. Jenniskens, M. Zolensky, *Meteorit. Bull.* **46**, 1 (2012).
- Materials and methods are available as supplementary materials on Science Online.
- D. R. Christie, P. Campus, in *Infrasound Monitoring for Atmospheric Studies*, A. Le Pichon, E. Blanc, A. Hauchecorne, Eds. (Springer, Dordrecht, Netherlands, 2010), pp. 29–75.
- T. A. Ens, P. G. Brown, W. N. Edwards, E. A. Silber, *J. Atmos. Sol. Terr. Phys.* **80**, 208 (2012).
- E. A. Silber, A. Le Pichon, P. Brown, *Geophys. Res. Lett.* **38**, L12201 (2011).
- M. H. Shaddad *et al.*, *Meteorit. Planet. Sci.* **45**, 1557 (2010).
- K. D. Smith *et al.*, *Science* **305**, 1277 (2004).
- H. Haack *et al.*, *Meteorit. Planet. Sci.* **47**, 30 (2012).
- H. Haack *et al.*, in *Workshop on the First Solids in the Solar System*, 7 to 9 November 2011, Koloa, Kauai, Hawai'i (Lunar and Planetary Institute, Houston, TX, 2011), abstr. 9100.
- K. Nishiizumi, M. W. Caffee, *Meteorit. Planet. Sci.* **44** (Suppl.), 5358 (2009).
- U. Ott, *Rev. Mineral. Geochem.* **47**, 71 (2002).
- V. S. Heber *et al.*, *Geochim. Cosmochim. Acta* **73**, 7414 (2009).
- I. Leya, H.-J. Lange, S. Neumann, R. Wieler, R. Michel, *Meteorit. Planet. Sci.* **35**, 259 (2000).
- M. Gounelle *et al.*, in *The Solar System Beyond Neptune*, M. A. Barucci, H. Boehnhardt, D. P. Cruikshank, A. Morbidelli, Eds. (Univ. of Arizona Press, Tucson, AZ, 2008), pp. 525–541.
- D. Nesvorný *et al.*, *Astrophys. J.* **713**, 816 (2010).
- K. J. Walsh, M. Delbo, W. F. Bottke, paper presented at the 44th American Astronomical Society/Division of Planetary Sciences Meeting, Reno, NV, 14 to 19 October, 2012, abstr. 305.04.
- A. Tsuchiyama *et al.*, *Meteorit. Planet. Sci.* **44** (suppl.), 5189 (2009).
- O. Popova *et al.*, *Meteorit. Planet. Sci.* **46**, 1525 (2011).
- D. S. Ebel, M. L. Rivers, *Meteorit. Planet. Sci.* **42**, 1627 (2007).
- R. J. Macke, D. T. Britt, G. J. Consolmagno, *Meteorit. Planet. Sci.* **46**, 311 (2011).
- D. T. Britt *et al.*, paper presented at the Meteoritical Society Meeting, Cairns, Australia, 12 to 17 August 2012, abstr. 5250.
- M. S. Spiegel, R. C. Reedy, O. W. Lazareth, P. W. Levy, L. A. Slate, *J. Geophys. Res.* **91**, 483 (1986).
- P. Rochette *et al.*, *Meteorit. Planet. Sci.* **43**, 959 (2008).
- J. Gattacceca, P. Rochette, *Earth Planet. Sci. Lett.* **227**, 377 (2004).
- A. Bischoff, E. R. D. Scott, K. Metzler, C. A. Goodrich, in *Meteorites and the Early Solar System II*, D. S. Lauretta, H. Y. McSweeney Jr., Eds. (Univ. of Arizona Press, Tucson, AZ, 2006), pp. 679–712.
- R. J. Walker *et al.*, *Geochim. Cosmochim. Acta* **66**, 4187 (2002).
- Q.-Z. Yin, K. Yamashita, A. Yamakawa, R. Tanaka, B. Jacobsen, D. S. Ebel, I. D. Hutcheon, and E. Nakamura, *Lunar Planet. Sci.* **XL**, abstr. 2006 (2009).
- J. M. Friedrich, M.-S. Wang, M. E. Lipschutz, *Meteorit. Planet. Sci.* **37**, 677 (2002).
- E. Tonui, M. E. Zolensky, M. E. Lipschutz, *Proc. NIPR Symp. Antarct. Meteorites* **15**, 38 (2002).
- M. Haq, F. A. Hasan, D. W. G. Sears, C. B. Moore, C. F. Lewis, *Geochim. Cosmochim. Acta* **53**, 1435 (1989).
- G. D. Cody *et al.*, *Earth Planet. Sci. Lett.* **272**, 446 (2008).
- E. D. Young, R. D. Ash, P. England, D. Rumble 3rd, *Science* **286**, 1331 (1999).
- G. K. Benedix, L. A. Leshin, J. Farquhar, T. Jackson, M. H. Thiems, *Geochim. Cosmochim. Acta* **67**, 1577 (2003).
- M. M. Grady, *Astron. Geophys.* **50**, 4.21 (2009).
- P. Schmitt-Kopplin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 2763 (2010).
- G. W. Cooper, W. M. Onwo, J. R. Cronin, *Geochim. Cosmochim. Acta* **56**, 4109 (1992).
- A. A. Monroe, S. Pizzarello, *Geochim. Cosmochim. Acta* **75**, 7585 (2011).
- D. P. Glavin, M. P. Callahan, J. P. Dworkin, J. E. Elsila, *Meteorit. Planet. Sci.* **45**, 1948 (2010).
- J. R. Cronin, S. Pizzarello, *Geochim. Cosmochim. Acta* **54**, 2859 (1990).
- K. Lodders, *Astrophys. J.* **591**, 1220 (2003).
- E. Jarosewich, R. S. Clarke Jr., J. N. Barrows, Eds., *Smithson. Contrib. Earth Sci.* **27**, 1 (1986).
- P. G. Brown *et al.*, *Science* **290**, 320 (2000).
- R. N. Clayton, T. K. Mayeda, *Geochim. Cosmochim. Acta* **63**, 2089 (1999).
- R. N. Clayton, T. K. Mayeda, J. N. Goswami, E. J. Olsen, *Geochim. Cosmochim. Acta* **55**, 2317 (1991).
- R. N. Clayton, T. K. Mayeda, A. E. Rubin, *J. Geophys. Res.* **89**, C245 (1984).
- G. J. MacPherson *et al.*, *Geochim. Cosmochim. Acta* **73**, 5493 (2009).
- E. D. Young, S. S. Russell, *Science* **282**, 452 (1998).

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S36
Tables S1 to S22
References (49–105)

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The Evolutionary Landscape of Alternative Splicing in Vertebrate Species

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How species with similar repertoires of protein-coding genes differ so markedly at the phenotypic level is poorly understood. By comparing organ transcriptomes from vertebrate species spanning ~350 million years of evolution, we observed significant differences in alternative splicing complexity between vertebrate lineages, with the highest complexity in primates. Within 6 million years, the splicing profiles of physiologically equivalent organs diverged such that they are more strongly related to the identity of a species than they are to organ type. Most vertebrate species-specific splicing patterns are cis-directed. However, a subset of pronounced splicing changes are predicted to remodel protein interactions involving trans-acting regulators. These events likely further contributed to the diversification of splicing and other transcriptomic changes that underlie phenotypic differences among vertebrate species.

Vertebrate species possess diverse phenotypic characteristics, yet they share similar repertoires of coding genes (*1*).

Evolutionary changes in transcriptomes underlie structural and regulatory differences associated with species-specific characteristics. For

example, species-dependent mRNA and non-coding RNA (ncRNA) expression patterns have been linked to mutational changes in cis- and trans-acting regulatory factors, as well as to phenotypic differences (2–5). However, because organ-dependent mRNA expression levels within individual species have been largely conserved during vertebrate evolution (6, 7), it seems unlikely that changes in gene expression (GE)

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account for the majority of phenotypic diversity among vertebrates. Through the variable use of cis-acting RNA elements in exons and flanking introns that are recognized by trans-acting factors, different pairs of splice sites in primary transcripts can be se-

lected in a cell type-, condition-, or species-specific manner (8–15). Changes in alternative splicing (AS) may therefore represent a major source of species-specific differences (16–25). Here, we describe a genome-wide investigation of AS differences among physiologically equiv-

alent organs from vertebrate species spanning the major tetrapod lineages. **Evolution of alternative splicing complexity.** High-throughput RNA sequencing (RNA-Seq) data were collected from whole brain, forebrain cortex, cerebellum, heart, skeletal muscle, liver,

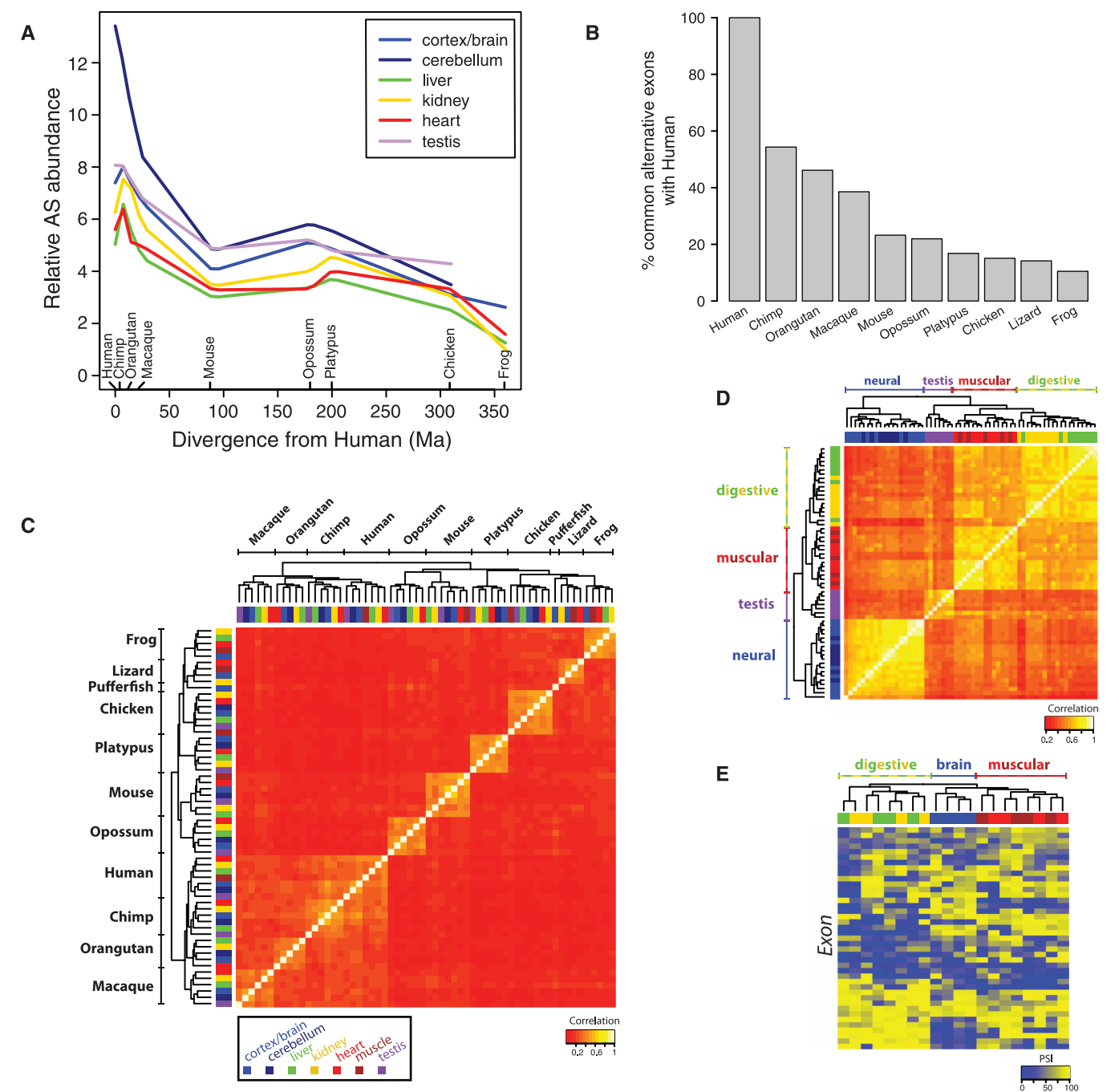


Fig. 1. Profiling of alternative splicing (AS) in vertebrates. **(A)** Relative proportions of exons undergoing AS in each sample, as measured by detection of middle exon skipping in random exon triplets, where the three exons are represented by orthologs in the analyzed species (y-axis units relative to the sample with lowest AS frequency). See fig. S1, A and B, for a more detailed version; see table S5 for details on samples, including replicates, and RNA-Seq data sets. **(B)** Percentage of common AS events between human and other species. **(C)** Symmetrical heat map of Spearman correlations from PSI profiles. For each sample, PSI values for the 1550

orthologous exons in the 11 analyzed species were estimated. See fig. S4A for a more detailed version. **(D)** Symmetrical heat map of Pearson correlations from gene expression (GE) profiles. For each sample, mRNA expression [log cRPKM values (26)] of 1809 analyzed orthologous genes in the 11 analyzed species were estimated. Key as in (C). See fig. S4B for a more detailed version. **(E)** Heat map of PSI values for 41 conserved cassette alternative exons. Rows, exons; columns, samples. Key as in (C). See fig. S11B for a more detailed version. Data are hierarchically clustered (complete method, Euclidean distance) for heat maps in (C) to (E).

kidney, and testis from human, chimpanzee, orangutan, macaque, mouse, opossum, platypus, chicken, lizard, and frog (26). For each species, we considered all internal exons as potential cassette AS events and created nonredundant databases of splice junction sequences formed by inclusion or skipping of each exon. RNA-Seq reads were mapped to the junction databases to determine “percent spliced-in” (PSI) values, and also to representative transcript sequences from each gene to estimate GE levels, represented as “corrected reads per kilobase transcript model per million mapped reads” (cRPKM) values (26). Orthology relationships between genes and exons were established to enable direct cross-species comparisons.

The relative proportions of orthologous exons detected to undergo AS in each sample were determined. Equal numbers of reads were randomly sampled from each RNA-Seq data set to control for coverage differences (26). AS detection is approximately twice as frequent in all analyzed pri-

mate organs as in the equivalent organs from mouse and other species (Fig. 1A and fig. S1). Moreover, there is an overall decline in AS frequency as the evolutionary distance from primates increases. These differences are significant ($P < 10^{-10}$, Mann-Whitney U tests), are robust to different methods of AS frequency detection, and are independent of the variability in AS detection rates between individuals within the same species (Fig. 1A and fig. S1). Genes with the highest AS complexity in human are significantly enriched in cytoskeleton-associated functions ($P < 0.03$) (table S1), which suggests that AS-directed diversification of the cytoskeleton may have been a driving force in the evolution of increased cellular complexity in vertebrate species.

Rapid evolution of organ-specific alternative splicing. We next compared AS profiles across organs and species. Approximately half of alternatively spliced exons among species separated by ~6 million years of evolution are different

(Fig. 1B and figs. S2A and S3A). When clustering organ AS profiles on the basis of how overall PSI values correlate in pairwise comparisons, the samples segregate by individual species (Fig. 1C and fig. S4A). This is in contrast to clustering samples on the basis of how their overall GE levels correlate, where the samples segregate according to tissue type (Fig. 1D and fig. S4B) (6, 7). Principal components analysis confirms that species type and tissue type are the primary sources of variability underlying the overall AS and GE patterns, respectively (figs. S5 and S6). The species-dependent clustering of AS profiles is also observed when analyzing subsets of alternative exons associated with a wide range of splice site strengths, exon length, increased magnitudes of PSI change between tissues, increased read coverage, and also when using independently validated (16) PSI differences (figs. S7 to S10). These results indicate that overall organ-specific AS patterns have evolved at a much more rapid rate than organ-specific GE patterns.

However, when restricting the clustering analysis to all ($n = 41$) orthologous exons that are alternatively spliced in four species (human, mouse, chicken, and frog) representing the main tetrapod lineages, the samples segregate by tissue type, with similar results obtained with the larger data set of tissues and species (Fig. 1E and fig. S11, A to H). The 41 exons, on average, display a wider range of inclusion levels across the samples, indicating that they have a higher degree of regulatory potential (fig. S11I). Consistent with this observation, they are also associated with elevated exonic and flanking intronic sequence conservation, implying that they are under increased selection pressure to maintain binding sites for regulatory trans-acting factors (26). Therefore, although overall AS patterns of multiple organs distinguish vertebrate species, a small subset of exons that undergo AS in multiple species spanning ~350 million years of evolution display conserved patterns of regulation that reflect organ type.

We next investigated the relative rates at which AS and GE have evolved. From pairwise comparisons of PSI values in homologous tissues, we observe an overall increase of PSI divergence from human with evolutionary time (Fig. 2A). In contrast to results from analyzing GE divergence (Fig. 2, C and D) (6, 7), AS levels in testis have not diverged more rapidly than in other tissues (Fig. 2B), and AS events detected in neural tissues display the slowest rate of divergence ($P < 10^{-6}$, Mann-Whitney U tests). A significantly higher proportion (27% more on average) of neural AS events are conserved between vertebrate species than are AS events specific to other organs ($P < 0.002$, Mann-Whitney U test; fig. S2B). These AS events are enriched in genes associated with synaptic transmission, axon guidance, neural development, and actin cytoskeleton reorganization (table S2), indicating that AS regulation of these processes is a highly conserved feature of vertebrate nervous system development.

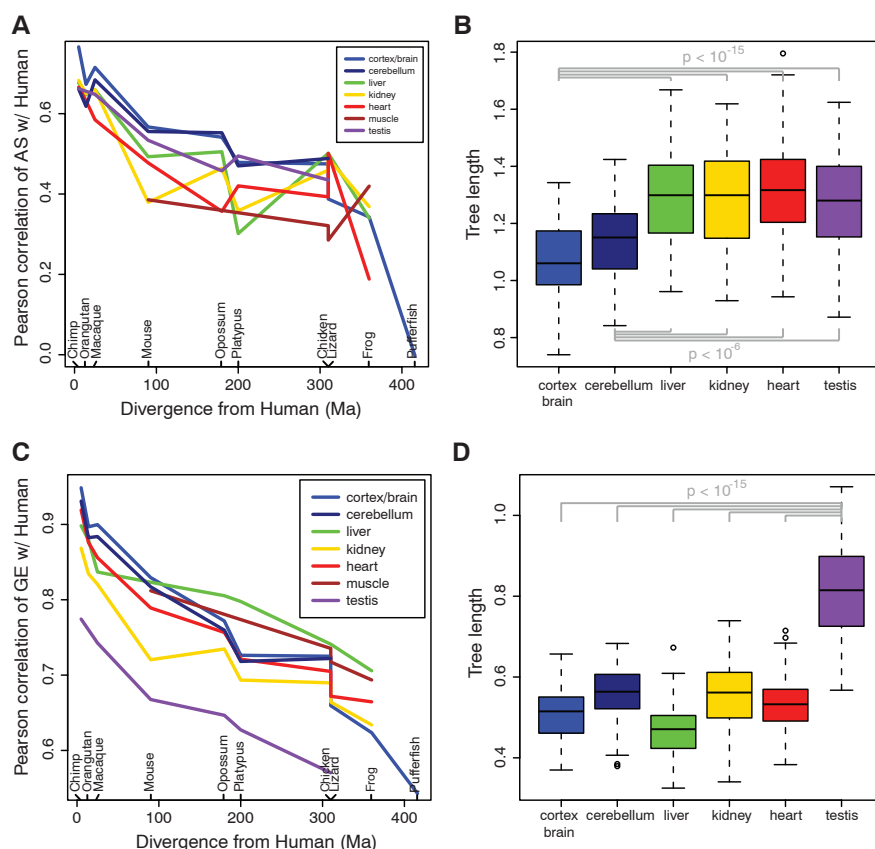


Fig. 2. Different rates of AS and GE divergence. (A) Pearson correlations between human and other species when comparing PSI values pairwise for conserved tissue-specific alternative exons in each tissue. For each pair of samples, correlation is analyzed for PSI values for all exons undergoing AS in both samples. (B) Comparisons of total tree lengths (bootstrapping, 100 replicates) of PSI trees for conserved tissue-specific alternative exons from six tissues in seven species (human, chimp, macaque, mouse, opossum, platypus, chicken). Statistically significant differences between the neural and each of the other tissues are indicated (Mann-Whitney U tests). Full PSI trees for each tissue are shown in fig. S3B. (C) Pearson correlations between human and other species when comparing GE pairwise (cRPKM, log scale; 1809 orthologous genes in the 11 analyzed species) in each organ. (D) Comparisons of total tree lengths (bootstrapping, 100 replicates) of expression trees from analyzing six organs from seven species as in (B). Statistically significant differences between testis and each of the other tissues are indicated (Mann-Whitney U tests). Full expression trees for each tissue are shown in fig. S3C.

Evolution of vertebrate splicing codes. To investigate mechanisms underlying the divergence in organ AS profiles, we used a splicing code derived from mouse data (27, 28) to compare cis-regulatory elements that are predictive of tissue-dependent splicing patterns of five organs (brain, heart, skeletal muscle, liver, and kidney) from the four representative species analyzed above. The splicing code achieved high classification rates when predicting organ-specific AS patterns from sequence alone. The average true positive rate [AUC, area under the receiver operating characteristic (ROC) curve] ranges from 68 to 70% for heart exon skipping to 80 to 88% for brain exon inclusion (Fig. 3, A and B, and fig. S12). An exception was a subset of approximately 200 exon-skipping AS events in human brain,

which were predicted less well (AUC = 62%) (fig. S12) (26). A comparison of the most strongly predictive cis-elements accounting for each species' organ-dependent splicing patterns revealed that these significantly overlap in most tissues (Fig. 3C and fig. S12). Such cis-elements may represent features comprising an ancestral vertebrate splicing code (Fig. 3D). However, the overlap between sets of splicing code features used in a given pair of species decreases with increased evolutionary distance (Fig. 3C and fig. S12; see also below). Thus, organ-dependent AS patterns appear to be generally controlled by significantly overlapping cis-regulatory codes, although progressive divergence in these codes likely also contributes to AS differences.

Species-specific alternative splicing is primarily cis-directed. The extent to which evolutionary change in cis-regulatory codes (versus trans-acting factors) accounts for species-dependent AS differences is not known. To address this, we used a mouse strain, Tc1, carrying the majority of human chromosome 21 (HsChr21) (29). We compared PSI values of exons from HsChr21 transcripts expressed in multiple organs (brain, liver, heart, testis) from the Tc1 strain, with PSI values of the identical exons in the corresponding human organs. We also compared PSI values for the orthologous mouse exons between wild-type and Tc1 mouse strains. For all comparisons, we analyzed a comprehensive set of 13 HsChr21 and orthologous mouse exons that were detected, using RNA-Seq data, to

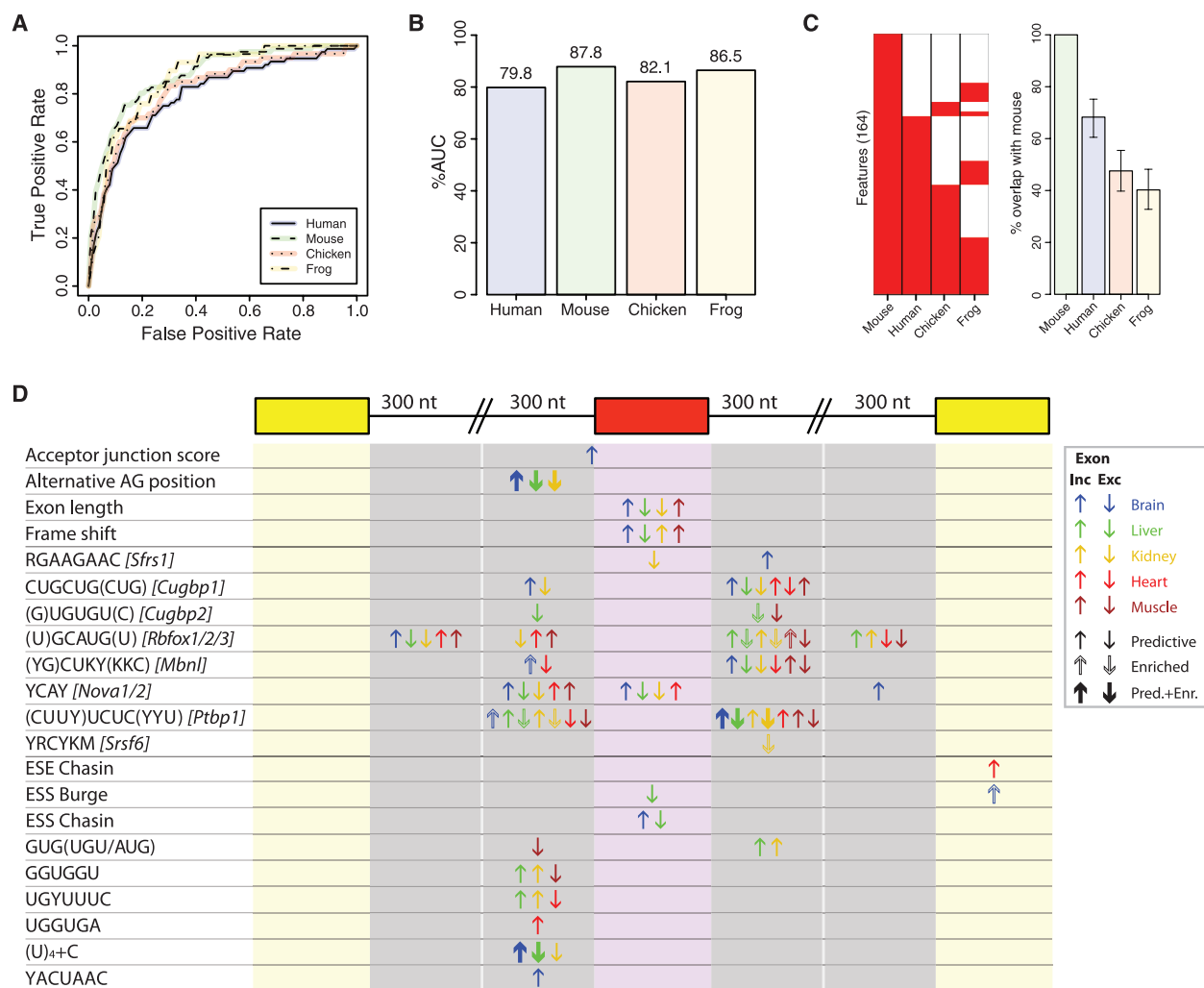


Fig. 3. Inference and comparative analysis of vertebrate splicing codes. (A) ROC curves of splicing code predictions for brain-dependent exon inclusion in human, mouse, chicken, and frog. ROC curves for other tissues are shown in fig. S12. (B) AUC in percent for each of the ROC curves in (A). (C) Left: Heat map of splicing code features with significant prediction scores associated with brain-specific exon inclusion in each species (red for $P < 0.05$, Mann-Whitney U test); statistically significant features in common with all significant mouse features associated with brain-specific exon inclusion are shown. Right: Proportions of features significantly associated with mouse brain-specific exon inclusion also significant for brain-specific exon inclusion in other species.

Error bars: 95% confidence intervals, Pearson's χ^2 proportion tests. Analyses of predictive features for other tissues are shown in fig. S12. (D) Region-specific distribution of code features significantly associated with tissue-specific exon inclusion or exclusion in all four species, as determined by their region-specific enrichment or by their predictive power as inferred by the splicing code. Splicing factors associated with code features are in square brackets. Significantly enriched features are indicated by hollow arrows; features both significantly enriched and predictive are indicated by bold arrows. Arrows are colored according to the organ with which the features are associated (refer to key).

have undergone AS in at least one of the two species.

All analyzed HsChr21 exons that are alternatively spliced in human for which the orthologous exons in mouse are constitutively spliced are also alternatively spliced in the Tc1 mouse; likewise, all HsChr21 constitutively spliced exons for which the orthologous exons in mouse are alternatively spliced are also constitutively spliced in the Tc1 mouse ($P < 0.02$, both comparisons; one-sided Fisher's exact tests; Fig. 4, A and B, and fig. S13A). For the orthologous exons that are alternatively spliced in human and mouse, we observe a significantly higher correlation between their inclusion levels in Tc1 mouse and normal human organs relative to the correlation observed when comparing inclusion levels of all orthologous human and mouse exons ($r = 0.89$ versus $r = 0.52$, $P < 0.0008$, one-sided Z-test; fig. S13B) (26).

Collectively, the results indicate that changes among predominantly conserved cis-regulatory elements are sufficient to direct the majority of the species-specific AS patterns, at least in human and mouse. However, because our results also indicate that vertebrate splicing codes diverged with increasing evolutionary distance, and because specific subsets of AS events could not be reliably predicted using the splicing code, changes in trans-acting factors likely also contributed to evolutionary differences in AS.

Species-classifying alternative splicing events in trans-acting regulatory factors. To investigate which splicing changes during evolution likely had the greatest functional impact, we identified AS events that best discriminate or “classify” species (26). These AS events have relatively large and widely expressed PSI differences between species or lineages, and were validated at a high rate by reverse transcription polymerase chain reaction (RT-PCR) assays ($r = 0.90$, $n = 180$; fig. S14). Gene Ontology enrichment analysis reveals that the corresponding genes are functionally diverse, with “nucleic acid binding” among the most frequently represented categories (Fig. 5A, fig. S14, and tables S3 and S4). Consistent with a major role for conserved cis-regulatory elements governing species-dependent AS profiles (Figs. 3 and 4), the species-classifying AS events are significantly underrepresented in exons that overlap nucleic acid binding domains, relative to other classes of alternative exons in the same genes ($P < 0.04$, Pearson's χ^2 proportion test; Fig. 5C).

An interesting example of a species-classifying AS event is the activity-modulating exon 9 of the splicing regulator PTBP1, located between RNA recognition motifs 2 and 3 in this protein (30, 31). This exon is skipped in mammalian organs but is fully included in chicken and frog organs (Fig. 5A). Consistent with the possibility of a more

variable and extensive regulatory role for PTBP1 in mammalian-specific AS, we observe significant enrichment of putative PTBP1 binding sites in sequences surrounding mammalian-specific AS events relative to sequences surrounding chicken- and frog-specific AS events ($P < 0.002$, one-sided Fisher's exact test; Fig. 5B).

The species-classifying AS events in genes associated with other functions are also significantly underrepresented in exons that overlap folded protein domains ($P < 0.0002$, Pearson's χ^2 proportion test; fig. S15A). However, the species-classifying AS events are significantly enriched in frame-preserving exons relative to nonclassifying species-specific AS events ($P \leq 0.05$, Pearson's χ^2 proportion test; Fig. 5D and fig. S15B), and they are also enriched in protein regions predicted to be disordered. Disordered regions generally reside on protein surfaces and are known to play critical roles in ligand interactions and cell signaling (32, 33), and recent work has shown that tissue-regulated exons enriched in predicted disordered sequences often function in remodeling protein-protein interactions (PPIs) (34, 35). The species-classifying AS events thus possess multiple features of functionally important exons.

Discussion. Our results show that organs of primate species have significantly higher cassette exon AS frequencies than do organs of other vertebrate species. Moreover, overall organ AS

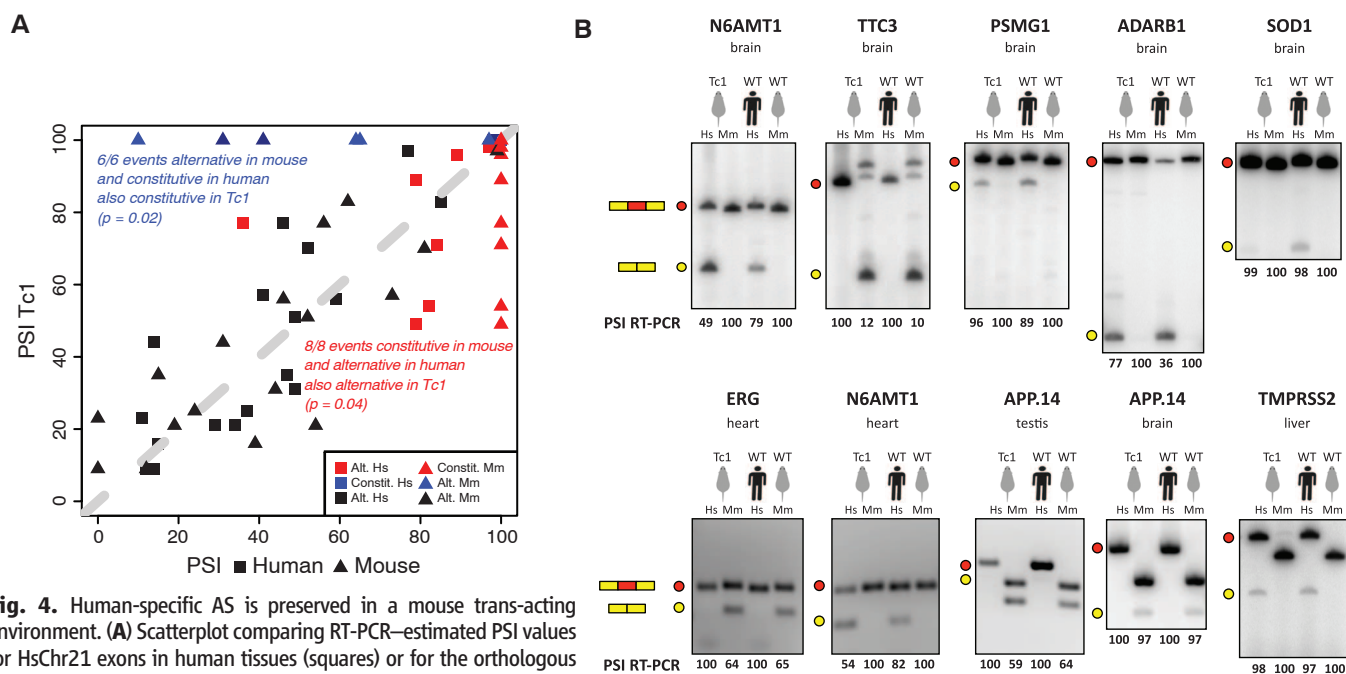


Fig. 4. Human-specific AS is preserved in a mouse trans-acting environment. **(A)** Scatterplot comparing RT-PCR-estimated PSI values for HsChr21 exons in human tissues (squares) or for the orthologous exons in the corresponding wild-type mouse tissues (triangles) (x axis), and the same HsChr21 exons in Tc1 mouse tissues (y axis) [13 different pairs of orthologous exons, 31 total pairs of orthologous AS events (26)]. Data points are colored according to whether the represented splicing events are alternative in human and constitutive in mouse (red), constitutive in human and alternative in mouse (blue), or alternative in both species (black) (26). Dark blue: exons with PSI > 95% and < 100% in human and PSI < 50% in mouse. Identity line is in dashed gray. P values for one-sided Fisher's exact tests are indicated. **(B)** RT-PCR experiments measuring PSI levels for pairs of orthologous human and mouse exons using species-specific primer pairs (26), for exons

alternative in one species and constitutive or near-constitutive (PSI > 95%) in the other. Human Chr21 exons were analyzed in Tc1 mouse tissues and normal human tissues; the orthologous mouse exons were analyzed in corresponding tissues from both the Tc1 and wild-type mouse strains. Quantification of PSI levels, human gene names (followed by the exon number, when more than one exon for the same gene was studied), and tissues are indicated. Red and yellow dots indicate exon-included and exon-skipped isoforms, respectively. Note that each set of species-specific primers amplifying the orthologous splice isoforms generates size-distinct RT-PCR products.

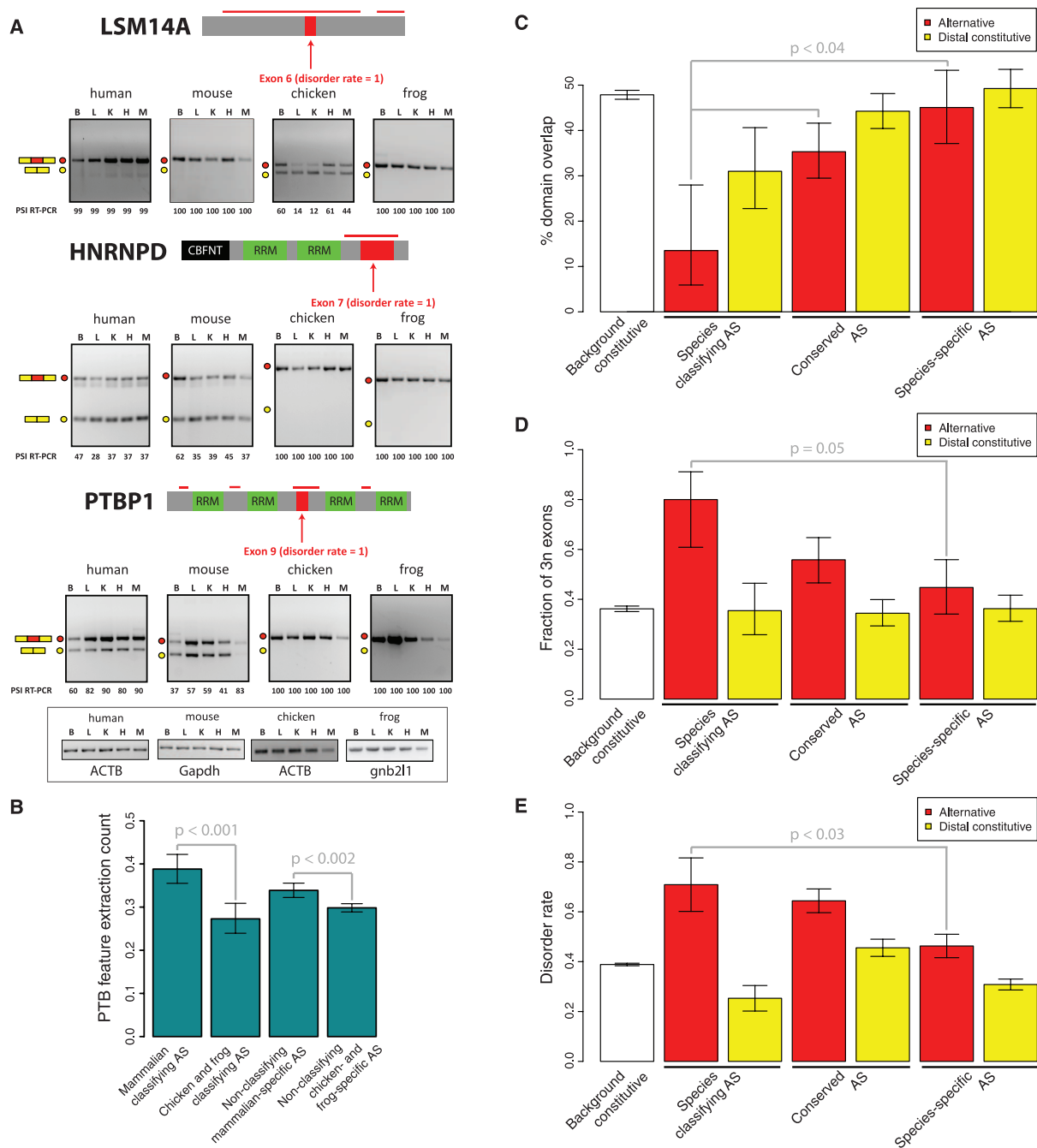


Fig. 5. Characterization of species-classifying AS events. **(A)** Domain diagrams and RT-PCR experiments measuring PSI levels for representative species-classifying AS events. Predicted highly disordered protein regions are depicted by red bars above the domain diagrams. Human gene names are indicated, as are the locations of the classifying alternative exon, species, and organs (B, brain; L, liver; K, kidney; H, heart; M, muscle) in which the validations were performed, as well as quantification of PSI levels. Red and yellow dots indicate exon-included and exon-skipped isoforms, respectively. RT-PCR assays were performed as in Fig. 4. **(B)** Comparisons of the average number of PTBP1-related cis-features associated with mammalian-classifying and nonclassifying species-dependent AS events, and the corresponding classes of species-dependent AS events in chicken and frog. Error bars: 95% confidence intervals, Pearson's χ^2 proportion tests. Statistically significant differences are indicated. **(C)** Comparison of the proportion of residues in different types of coding exons in proteins with nucleic acid-binding domains that overlap folded protein domains. Classes of alternative exons (red bars) analyzed are (i) "species-classifiers," AS events that discriminate species; (ii) "conserved AS," exons detected as AS in at least two of the analyzed species; and

(iii) "species-specific AS," exons that are alternatively spliced in only one of the analyzed species. For each class of alternative exon, distal constitutive exons (separated from the alternative exon by at least two exons) in the same genes are analyzed (yellow bars). "Background constitutive" refers to all exons that are constitutively spliced in all tissues (white bars). Error bars: 95% confidence intervals, Pearson's χ^2 proportion tests. Statistically significant differences between species-classifying AS events and each of the other classes of AS events are indicated. **(D)** Proportion of exons in proteins with nucleic acid binding domains that preserve the reading frame when included/skipped in transcripts (3n exons, exons with nucleotide length multiple of three nucleotides). Types of exons analyzed are as in (C). Error bars: 95% confidence intervals, Pearson's χ^2 proportion tests. Statistically significant differences between species-classifying AS events and the other species-specific AS events are indicated. **(E)** Proportion of predicted intrinsically disordered amino acids in different classes of exons in proteins with nucleic acid binding domains, as described in (C). Error bars denote SE of the associated distributions. Statistically significant differences between species-classifying AS events and the other species-specific AS events are indicated (Student's t test).

profiles more strongly reflect the identity of a species than they do organ type. This contrasts with organ-dependent differences in mRNA expression, which are largely conserved throughout vertebrate evolution [this study and (6, 7)]. We propose that the rapid divergence in AS patterns in vertebrate organs may have played a more widespread role in shaping species-specific differences than did changes in mRNA expression.

Our work offers conclusive evidence that the reassortment of splicing code features can account for the majority of AS differences between vertebrate species. Consistent with this observation, species-classifying exons identified in this study are often found in genes encoding trans-acting regulators but are underrepresented in the nucleic acid binding domains of these proteins. Instead, they are highly enriched in disordered regions, which are known to function in signaling and in mediating PPIs. Because these AS changes affect trans-acting factors involved in gene regulation, they represent an additional mechanistic basis for the remarkable diversification in AS and other transcriptomic changes associated with phenotypic change among vertebrate species.

References and Notes

1. C. P. Ponting, *Nat. Rev. Genet.* **9**, 689 (2008).
2. A. M. Heimberg, L. F. Sempere, V. N. Moy, P. C. Donoghue, K. J. Peterson, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2946 (2008).
3. V. J. Lynch, G. P. Wagner, *Evolution* **62**, 2131 (2008).

4. A. C. Meireles-Filho, A. Stark, *Curr. Opin. Genet. Dev.* **19**, 565 (2009).
5. P. J. Wittkopp, G. Kalay, *Nat. Rev. Genet.* **13**, 59 (2011).
6. D. Brawand *et al.*, *Nature* **478**, 343 (2011).
7. E. T. Chan *et al.*, *J. Biol.* **8**, 33 (2009).
8. A. N. Brooks *et al.*, *Genome Res.* **21**, 193 (2011).
9. J. C. Castle *et al.*, *Nat. Genet.* **40**, 1416 (2008).
10. M. Irimia, B. J. Blencowe, *Curr. Opin. Cell Biol.* **24**, 323 (2012).
11. N. Jelen, J. Ule, M. Živin, R. B. Darnell, *PLoS Genet.* **3**, e173 (2007).
12. A. Kalsotra, T. A. Cooper, *Nat. Rev. Genet.* **12**, 715 (2011).
13. H. Keren, G. Lev-Maor, G. Ast, *Nat. Rev. Genet.* **11**, 345 (2010).
14. D. D. Licatalosi, R. B. Darnell, *Nat. Rev. Genet.* **11**, 75 (2010).
15. T. W. Nilsen, B. R. Graveley, *Nature* **463**, 457 (2010).
16. J. A. Calarco *et al.*, *Genes Dev.* **21**, 2963 (2007).
17. S. Gelfman *et al.*, *Genome Res.* **22**, 35 (2012).
18. E. O. Gracheva *et al.*, *Nature* **476**, 88 (2011).
19. M. Irimia, J. L. Rukov, S. W. Roy, J. Vinther, J. Garcia-Fernandez, *Bioessays* **31**, 40 (2009).
20. G. Lev-Maor, R. Sorek, N. Shomron, G. Ast, *Science* **300**, 1288 (2003).
21. B. Modrek, C. J. Lee, *Nat. Genet.* **34**, 177 (2003).
22. Q. Pan *et al.*, *Trends Genet.* **21**, 73 (2005).
23. Y. Terai, N. Morikawa, K. Kawakami, N. Okada, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12798 (2003).
24. G. W. Yeo, E. Van Nostrand, D. Holste, T. Poggio, C. B. Burge, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2850 (2005).
25. X. H. Zhang, L. A. Chasin, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 13427 (2006).
26. See supplementary materials on Science Online.
27. Y. Barash *et al.*, *Nature* **465**, 53 (2010).
28. H. Y. Xiong, Y. Barash, B. J. Frey, *Bioinformatics* **27**, 2554 (2011).
29. A. O'Doherty *et al.*, *Science* **309**, 2033 (2005).

30. A. Corriero, J. Valcárcel, *Mol. Cell* **36**, 918 (2009).
31. M. C. Wollerton *et al.*, *RNA* **7**, 819 (2001).
32. J. Bellay *et al.*, *Mol. Biosyst.* **8**, 185 (2012).
33. A. K. Dunker, I. Silman, V. N. Uversky, J. L. Sussman, *Curr. Opin. Struct. Biol.* **18**, 756 (2008).
34. J. D. Ellis *et al.*, *Mol. Cell* **46**, 884 (2012).
35. M. Buljan *et al.*, *Mol. Cell* **46**, 871 (2012).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6114/1587/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S15
Tables S1 to S9
References (36–78)

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Evolutionary Dynamics of Gene and Isoform Regulation in Mammalian Tissues

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Most mammalian genes produce multiple distinct messenger RNAs through alternative splicing, but the extent of splicing conservation is not clear. To assess tissue-specific transcriptome variation across mammals, we sequenced complementary DNA from nine tissues from four mammals and one bird in biological triplicate, at unprecedented depth. We find that while tissue-specific gene expression programs are largely conserved, alternative splicing is well conserved in only a subset of tissues and is frequently lineage-specific. Thousands of previously unknown, lineage-specific, and conserved alternative exons were identified; widely conserved alternative exons had signatures of binding by MBNL, PTB, RBFOX, STAR, and TIA family splicing factors, implicating them as ancestral mammalian splicing regulators. Our data also indicate that alternative splicing often alters protein phosphorylatability, delimiting the scope of kinase signaling.

Alternative pre-mRNA processing can result in mRNA isoforms that encode distinct protein products, or may differ exclusively in untranslated regions, potentially affecting mRNA stability, localization, or translation (1). It can also produce nonfunctional mRNAs that are targets of nonsense-mediated mRNA decay, serving to control gene expression (2). Most human alternative splicing is tissue-regulated (3, 4), but the extent to which tissue-specific splicing patterns are conserved across

mammalian species has not yet been comprehensively studied.

To address outstanding questions about the conservation and functional importance of tissue-specific splicing, we conducted transcriptome sequencing (RNA-Seq) analysis of nine tissues from five vertebrates, consisting of four mammals and one bird. The species, chosen on the basis of the quality of their genomes (all high-coverage finished or draft genomes) and their evolutionary relationships, include the rodents mouse and rat,

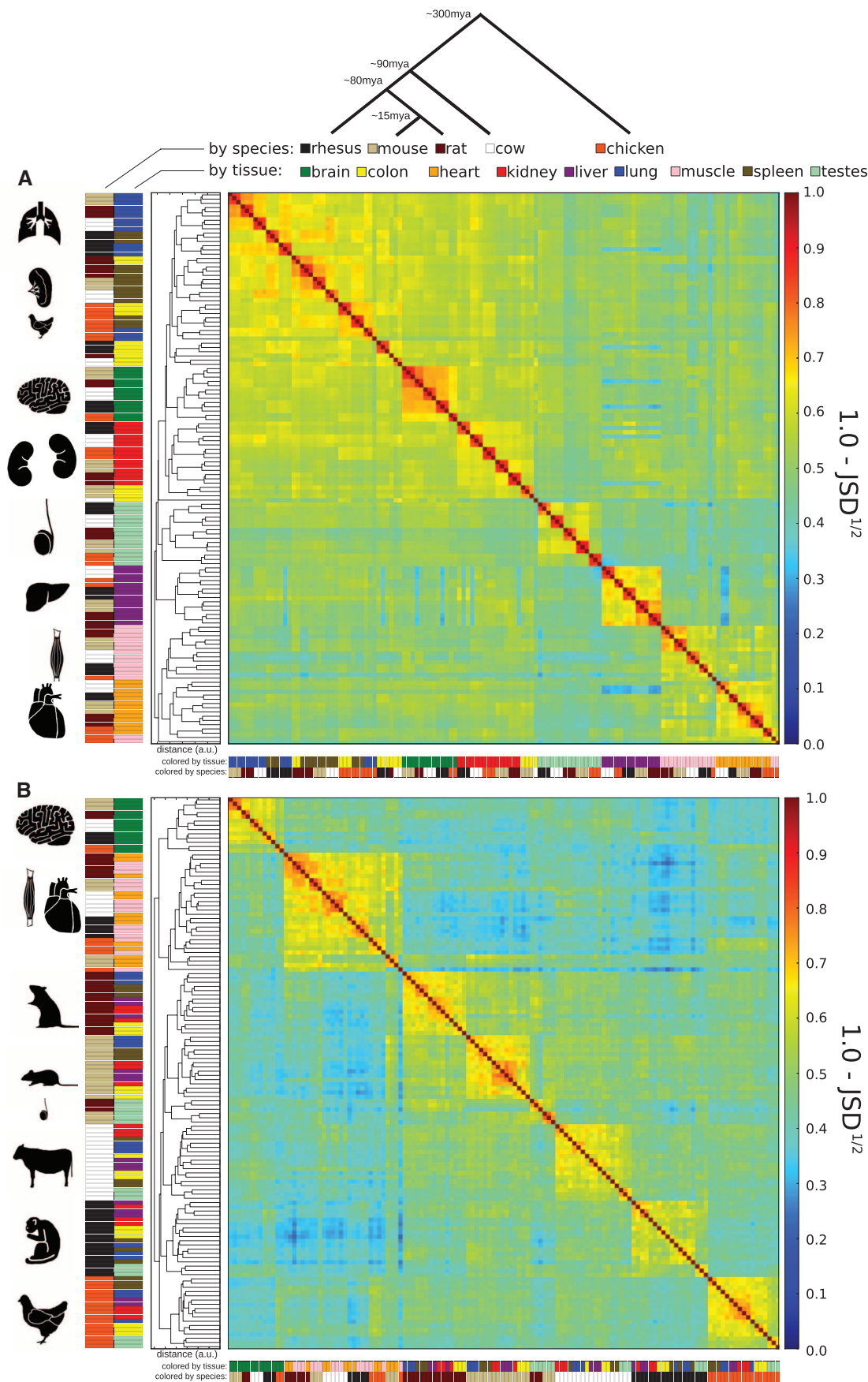
the rhesus macaque, a nonrodent/nonprimate boreutherian, cow, and chicken as an outgroup. These relationships allow for the evaluation of transcriptome changes between species with divergence times ranging from <30 million years to >300 million years (Fig. 1A). Our sequencing strategy used paired-end short or long read sequencing of poly(A)-selected RNA. In total, we generated more than 16 billion reads (>8 billion read pairs) totaling over 1 trillion bases (3, 5) (table S1). The data were mapped to the relevant genomes with software that can identify novel splice junctions and isoforms (6).

To assess coverage of genes, we compared these de novo annotations with existing Ensembl annotations. We detected >211,000 (97%) of the ~217,000 annotated exons in mouse, and similarly high fractions in most other species, including more than 99% of exons in chicken (table S1). We estimated that nearly all multi-exonic genes in the species studied are alternatively spliced (fig. S1) (3).

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Fig. 1. Conservation of expression signatures in all tissues and of alternative splicing signatures in some tissues. **(A)** Clustering of samples based on expression values, calculated as fragments per million mapped fragments per kilobase of mRNA (FPKM) of singleton orthologous genes present in all five species ($n = 7713$). Average linkage hierarchical clustering was used with distance between samples measured by the square root of the Jensen-Shannon divergence (JSD) between the vectors of expression values. **(B)** Clustering of samples based on PSI values of exons in singleton orthologous genes conserved to chicken, with alternative splicing detected in all individuals analyzed ($n = 489$). Clustered as in (A). When the set of genes used in this analysis was clustered by gene expression rather than PSI values, tissue-dominated clustering was observed, as in (A) (fig. S15).



All tissues have conserved expression signatures. To explore the expression relationships between the samples, we used hierarchical clustering based on Jensen-Shannon divergence (JSD) distances between the expression of orthologous genes. A clear pattern emerged in the resulting dendrogram (Fig. 1A and table S2). Samples of the same tissue from different individuals of the same species were invariably the most similar, followed by samples from the same tissue from other species, with few exceptions. This “tissue-dominated clustering” pattern indicates that most tissues possess a conserved gene expression signature and suggests that conserved gene expression differences underlie tissue identity in mammals (5, 7). Because gene expression varies by cell type, some observed differences could reflect changes in cell type composition. The most notable exceptions to tissue dominance were that some chicken muscle samples clustered with chicken heart rather than mammalian muscle, and that chicken lung, colon, and spleen samples clustered with each other rather than with their mammalian counterparts. These exceptions suggest that species-specific divergence in expression begins to exceed conserved tissue-specific differences at a phylogenetic distance of ~300 million years, corresponding to the split between birds and mammals.

Some tissues have conserved splicing signatures. To understand the splicing relationships between the samples, we performed an analogous clustering analysis using the “percent spliced in” (PSI or Ψ) values of the exons that were alternatively spliced in all species containing them. PSI values, the fraction of a gene’s mRNAs that contain the exon, were calculated from transcript abundance measurements (8) (fig. S2) and were clustered by using the same metric (Fig. 1B). Samples of the same tissue from individuals of the same species almost invariably clustered together. However, at larger distances, a more complex pattern emerged. Tissue-dominated clustering was observed for brain and for the combination of heart and muscle, indicating that these tissues have strong splicing signatures conserved between mammals and chicken, and the rodent testis samples also clustered together. By contrast, samples from the remaining tissues (colon, kidney, liver, lung, spleen) exhibited “species-dominated clustering,” forming distinct clusters by species rather than by tissue. This trend suggests that alternative splicing patterns specific to this latter group of tissues are less pronounced or less conserved than those of brain, testis, heart, and muscle (fig. S3). The greater prominence of species-dominated clustering of PSI values suggests that exon splicing is more often affected by lineage-specific changes in cis-regulatory elements (9) and/or trans-acting factors than is gene expression (6). Lineage-specific changes in splicing factor expression may have contributed to the tendency of splicing patterns to cluster by species more often than by tissue (table S3 and fig. S4).

Features of conserved, tissue-specific alternative exons. A subset of several hundred alternative exons exhibited highly conserved tissue-specific splicing patterns. The gene for eukaryotic translation elongation factor 1 delta (*EEF1d*) (Fig. 2A) and many other examples in our data demonstrate that highly tissue-specific patterns of splicing can be conserved for hundreds of millions of years (9).

To assess the phylogenetic distribution of alternative splicing events across mammals, we grouped exons by the inferred age of alternative splicing, defined as $\text{PSI} < 97\%$. Out of ~48,000 internal exons with clear orthologs in chicken and at least two mammals, we identified exons alternatively spliced in a species- or rodent-specific manner as well as ~500 “broadly alternative” exons with alternative splicing observed in all mammals studied (Fig. 2B and table S4). Conversely, we identified exons that were constitutively spliced in a lineage-specific manner (and alternatively spliced elsewhere), representing losses of alternative splicing. Using data from the Illumina human Body Map 2.0 data set, we found that rhesus-specific alternative exons were twice as likely to be detected as alternatively spliced in human as were exons with exon skipping detected only in a single rodent (fig. S5), consistent with the closer phylogenetic relationship of human to rhesus than to mouse or rat. In addition, more than 500 exons were identified whose phylogenetic splicing patterns imply multiple changes between constitutive and alternative splicing during mammalian evolution, suggesting frequent interconversion between constitutive and alternative splicing (10).

We observed a monotonic increase in tissue specificity within mouse as the phylogenetic breadth of alternative splicing increased from one to four mammals (Fig. 2, B and C). The fraction of exons that preserved reading frame in both inclusion and exclusion isoforms also increased from ~40 to ~70% with increasing phylogenetic breadth of alternative splicing. These patterns suggest that more broadly occurring (ancient) alternative splicing events function primarily to generate distinct protein isoforms, which are often tissue-specific (11). By contrast, more lineage-restricted (recently evolved) alternative splicing events appear to contribute more often to regulation involving reading frame disruption, which may yield truncated or nonfunctional mRNAs or proteins or serve to down-regulate expression, usually in a less tissue-specific manner.

Splice site changes may convert alternative to constitutive splicing. Exons that recently converted from constitutive to alternative splicing had significantly weaker 3' and 5' splice sites in the alternative splicing species than in their constitutively spliced orthologs (Fig. 2C) (10). However, recent conversion from alternative to constitutive splicing was not associated with significant changes in 5' or 3' splice site strength, suggesting involvement of other events such as loss of negative-acting cis-regulatory elements.

Constitutive exons that converted to alternative splicing in other species tended to have weaker splice sites than maintained constitutive exons ($P < 0.01$, rank-sum test), suggesting that exons with weaker splice sites may be predisposed to convert to alternative splicing. We found that exons with nearby G-runs [often bound by heterogeneous nuclear RNP H (hnRNP H) family proteins] were 25 to 60% more likely to have converted from constitutive to alternative splicing (fig. S6) (12).

Exons alternatively spliced in all mammals tended to have the weakest 5' and 3' splice sites, approximately 1 bit weaker than maintained constitutively spliced exons (Fig. 2C) (13). These exons had mean PSI values that were closer to 50% than other exon groups (Fig. 2C), suggesting that weaker splice sites may have evolved in these exons to enable a broader range of exon inclusion levels.

Splicing cis-regulatory elements located adjacent to (or within) alternative exons often confer regulation through interaction with cell type- or condition-specific protein factors (14). Using a large set of intronic splicing regulatory element (ISRE) motifs recognized by both tissue-specific and broadly expressed splicing factors derived from (15, 16), we observed reduced motif turnover in exons alternatively spliced in multiple species relative to constitutive or recently converted alternative exons (Fig. 2D) (11, 17). Exons that converted from alternative to constitutive splicing in one or both rodents showed substantially increased turnover of ISREs than mammalian-wide alternative exons (Fig. 2D), suggesting that mutations affecting ISREs may contribute to these conversions.

Tissue-specific regulatory motifs accumulate in broadly alternative exons. Using vertebrate whole-genome alignments, we observed strong sequence conservation of only the exon and core splice site motifs in broadly constitutive exons and exons that recently acquired alternative splicing. However, increased sequence conservation both within the exon and extending at least 70 bases into the intron on either side was observed with increasing phylogenetic breadth of alternative splicing (Fig. 3A), suggesting the occurrence of purifying selection on adjacent ISREs and providing support for the reliability of these exon classifications.

To assess the nature of potential regulatory elements present in introns adjacent to alternative exons, we ranked pentanucleotides (5mers) by their relative frequency of occurrence in introns downstream of broadly alternative exons relative to constitutive exons using an information criterion (6). Among the top 10 5mers in this ranking were perfect or near-perfect matches to consensus motifs for tissue-specific splicing regulatory factors, including those of the MBNL, PTB, RBFOX, STAR, and TIA families of splicing factors (18) (Fig. 3B and table S5). Presence of motifs associated with almost all of these splicing factor families was conserved downstream of

broadly alternative exons more than two standard deviations more often than control motifs (Fig. 3C), implying strong selection to maintain their presence. Pronounced enrichment of these motifs was restricted primarily to exons with broad alternative splicing (≥ 4 species), with only modest enrichment downstream of rodent-specific alternative exons and little or no enrichment near mouse-specific alternative exons (Fig. 3D and fig. S7). These observations suggested that exons with more ancient alternative

splicing—which are more often tissue-specific (Fig. 2B)—are more reliant for their regulation on a distinct subset of ISRE motifs corresponding to the tissue-specific factors listed above (MBNL, RBFOX, etc.). To explore this hypothesis, we analyzed cross-linking/immunoprecipitation-sequencing (CLIP-Seq) data to assess the transcriptome-wide binding of the mouse splicing factor muscleblind-like 1 (MBNL1) (19). Greater phylogenetic breadth of alternative splicing was associated with about

threefold-increased frequency of in vivo MBNL1 binding (Fig. 3E). Presence of an MBNL motif was associated with increased binding near alternative but not constitutive exons (Fig. 3F), suggesting that motif presence is necessary but not sufficient for strong binding in vivo. As a group, broadly alternative exons have somewhat higher density of MBNL motifs (Fig. 3B), but increased frequency of MBNL binding was observed even when comparing to subsets of constitutive or more narrowly alternative exons with identical MBNL

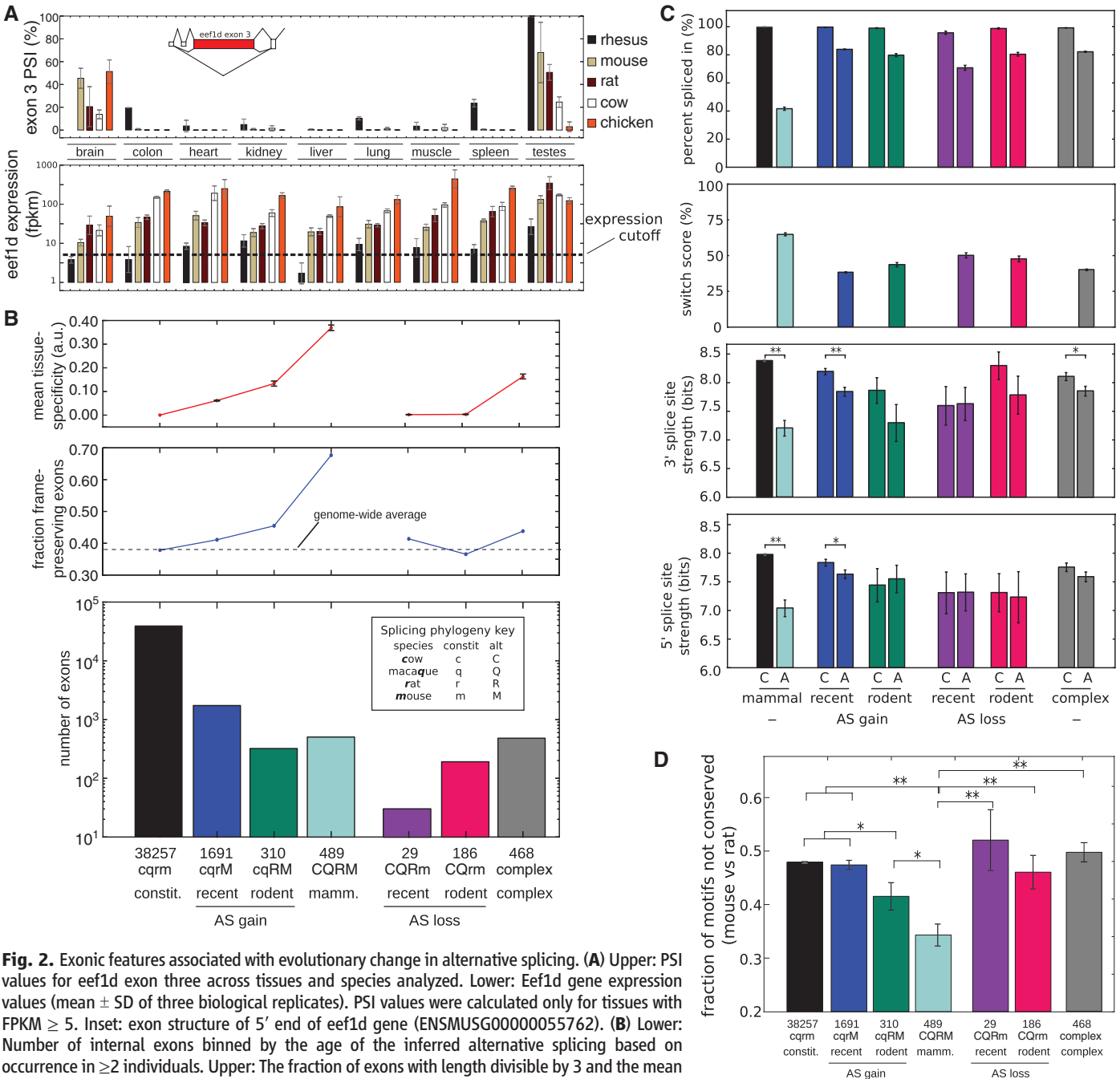


Fig. 2. Exonic features associated with evolutionary change in alternative splicing. **(A)** Upper: PSI values for *eef1d* exon three across tissues and species analyzed. Lower: *Eef1d* gene expression values (mean $\pm$ SD of three biological replicates). PSI values were calculated only for tissues with FPKM ≥ 5 . Inset: exon structure of 5' end of *eef1d* gene (ENSMUSG0000005762). **(B)** Lower: Number of internal exons binned by the age of the inferred alternative splicing based on occurrence in ≥ 2 individuals. Upper: The fraction of exons with length divisible by 3 and the mean and SEM of the tissue specificity. **(C)** Top: mean $\pm$ SEM of PSI values of exons binned by the phylogenetic extent of alternative splicing as in (B). Middle: mean $\pm$ SEM of 3' splice site scores of exons in each bin. Bottom: mean $\pm$ SEM of 5' splice site scores. Splice sites were scored with the MaxEnt model (31). * $P < 0.05$, ** $P < 0.001$ (Student's *t* test). **(D)** Fraction of regulatory 5mers in the downstream intron that differed between mouse and rat in exons binned by the phylogenetic extent of alternative splicing as in (B) (mean $\pm$ SEM). * $P < 0.05$, ** $P < 0.001$ (Student's *t* test).

motif counts (Fig. 3G). These observations suggest that broadly alternative exons have evolved features beyond motif abundance (such as favor-

able RNA structural features) to enhance binding of MBNL family splicing regulators. This phenomenon may extend to other factors (fig. S8).

Alternative splicing alters phosphorylation potential. Exons whose presence was widely conserved (at least four out of five species) were

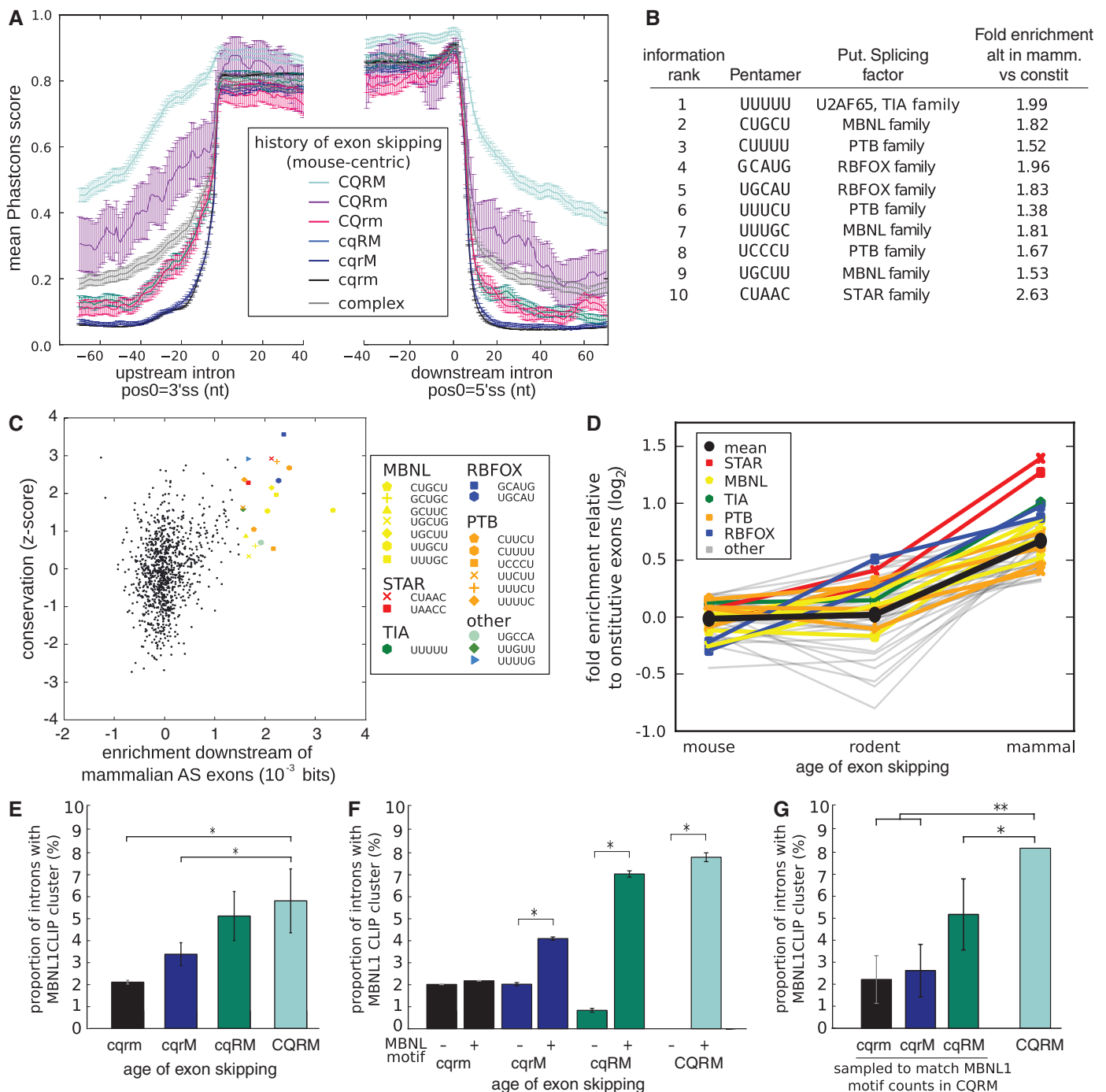


Fig. 3. Tissue-specific regulatory motifs accumulate and are preferentially bound in broadly alternative exons. **(A)** Mean $\pm$ SEM of Phastcons scores (using the placental mammals alignment, with mouse coordinates) in exons and flanking introns grouped by phylogenetic pattern of alternative splicing. Splicing pattern indicated by letters adjacent to colored bars, as in Fig. 2B. **(B)** Top 10 5mers in broadly alternative exons relative to constitutive exons ranked by discrimination information (6). **(C)** The conservation of all 5mers (6) compared with their discrimination information. All 5mers with discrimination information ≥ 0.001 bits are highlighted. UUUUU was an outlier in enrichment (0.011 bits) and is not shown. **(D)** Fold enrichment ($\log_2$) relative to constitutive exons in downstream region was plotted for 5mers

with discrimination information ≥ 0.001 bits for exons grouped by phylogenetic breadth of alternative splicing. The 5mers associated with known splicing regulators are shown in color, with mean of all 5mers in black. **(E)** The fraction of introns containing MBNL1 CLIP-Seq clusters (19) was assessed in introns adjacent to exons with different phylogenetic patterns of splicing, as in (A). **(F)** As in (E), but grouped by presence or absence of an MBNL1 motif. The mean fraction $\pm$ SEM of 1000 bootstrap samples is shown. $*P < 0.01$ (binomial test). **(G)** As in (E), but with exons sampled from each set to match the MBNL motif counts in the CQRM set. Mean $\pm$ SD of 1000 samplings is shown for the first three groups; observed mean is shown for the CQRM set. $*P < 0.05$, $**P < 0.001$.

classified on the basis of tissue specificity and evolutionary conservation of their splicing patterns with JSD-based metrics (6) into constitutive exons and four groups of alternative exons grouped by the degree of tissue specificity and evolutionary conservation of their splicing patterns. Functional analysis of species-specific alternative exons yielded few significant biases (table S6). However, analysis of the tissue-specific conserved group using DAVID (20, 21) identified a number of significantly enriched keywords and Gene Ontology (GO) categories, including several related to cell-cell junctions and cytoskeleton (Fig. 4A), suggesting that these splicing events may contribute to differences in cell structure, cell motility, and tissue architecture (22). The most enriched keyword, “alternative splicing,” reflects simply the abundant alternative splicing of this set of genes; the next most significantly enriched keyword was “phosphoprotein.”

To explore this connection to phosphorylation, we used Scansite (23) to predict phosphorylation sites in peptides encoded by different subsets of exons. Tissue-specific alternatively spliced exons, including both the conserved and nonconserved subsets, contained about 40% more predicted phosphorylation sites than other classes of exons (Fig. 4B and fig. S9). A comparable degree of enrichment for phosphorylation sites was observed in these exons with the curated PhosphoSite database (24) of experimentally determined phosphorylation sites (Fig. 4B). Phosphorylation site density in exons was correlated with phylogenetic breadth of alternative splicing (Fig. 4C and fig. S10). These observations suggest that tissue-specific alternative splicing is often used to alter the potential for protein phosphorylation, which can alter protein stability, enzymatic activity, subcellular localization, and other properties.

Exon 20 of the mouse tight junction protein 1 (TJP1) gene exhibits strongly tissue-specific alternative splicing and encodes a peptide containing an established phosphorylation site (25) that is predicted to be phosphorylated by ERK1 (also called MAPK3). In rhesus, ERK1 expression was above its median value in colon, lung, testis, and brain (therefore referred to as “kinase high” tissues) relative to liver, heart, muscle, and spleen (“kinase low” tissues). The PSI value of TJP1 exon 20 was much more variable in the kinase high tissues, ranging from 9% in testis to 90% in colon—a switch score of 81%—than in the kinase low tissues, where it had a switch score of 38%. Similar trends were observed in cow (Fig. 4D), and in rat and mouse (not shown).

To explore this phenomenon, we analyzed the splicing patterns of exons that contained predicted phosphorylation sites in relation to the expression of the associated kinases. To characterize the relationship between each exon-kinase pair, we defined the “kinase switch index” (KSI) as the switch score in “kinase high” tissues minus the switch score in “kinase low” tissues (see example in Fig. 4D). We used RNA-Seq estimates

of kinase expression, which were reasonably well correlated with in vivo kinase activity patterns ($r = 0.71$, fig. S11). We observed that

phosphorylation of sites within conserved, tissue-regulated exons is more tissue-specific than in other sets of exons (fig. S12) and that these exons

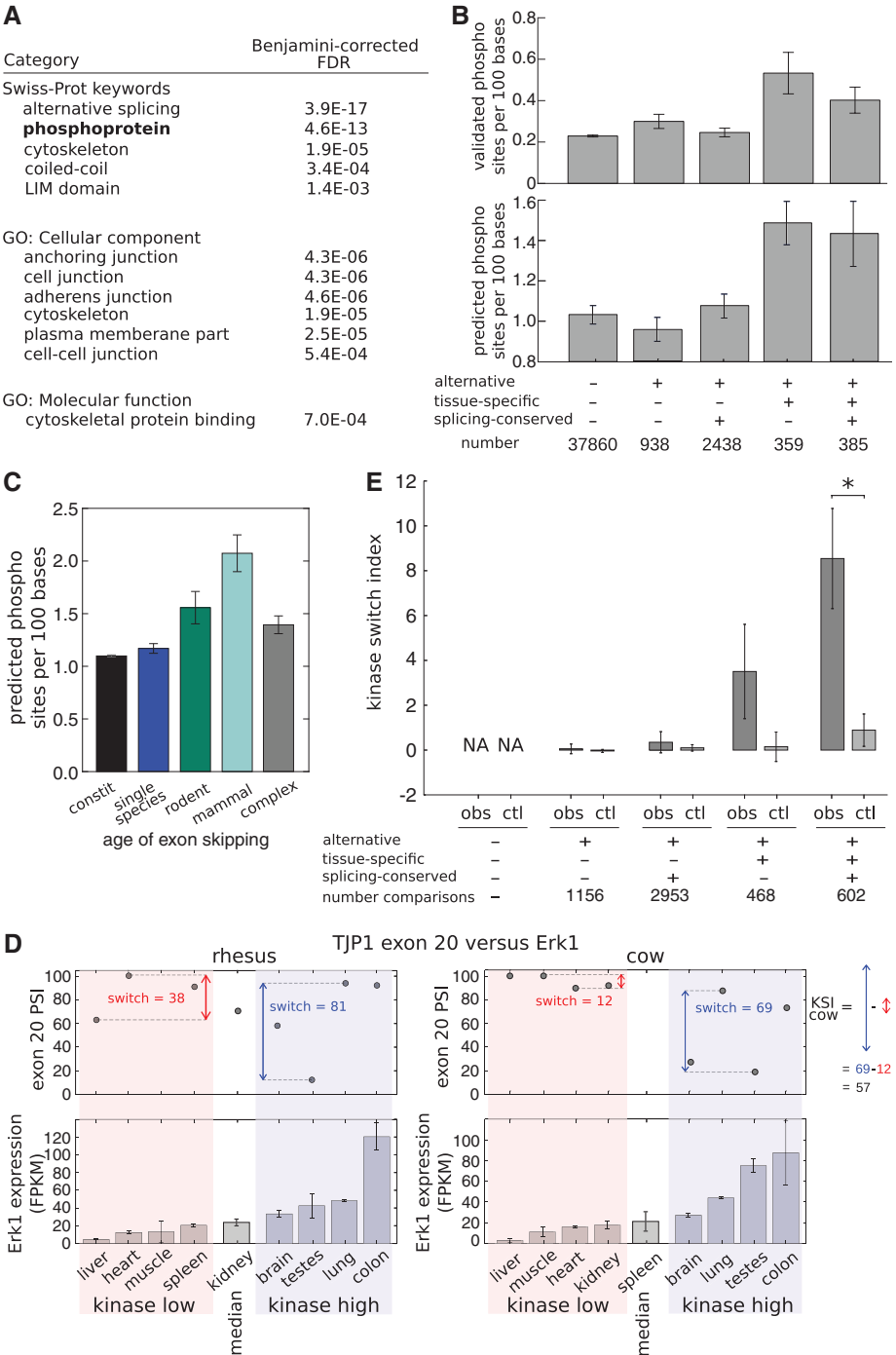


Fig. 4. Alternative splicing delimits the scope of protein phosphorylation. **(A)** GO analysis of genes containing tissue-regulated exons whose splicing is conserved. **(B)** Density of PhosphoSite phosphorylation sites (top) or Scansite-predicted phosphorylation sites (bottom) in exons grouped by alternative splicing status, tissue specificity, and splicing pattern conservation (6). Mean $\pm$ SEM is shown. **(C)** Mean Scansite-predicted phosphorylation site density in exons grouped by phylogenetic breadth of splicing. **(D)** TJP1 exon 20 splicing has a higher switch score in tissues where Erk1 is expressed above median levels (shaded blue) than where Erk1 is expressed below median (shaded pink); KSI is defined as the difference between these switch scores. PSI value was not calculated if TJP1 expression fell below a cutoff (e.g., cow spleen). **(E)** Mean KSI values for kinase-exon pairs involving the sets of exons as in (B). Mean $\pm$ SEM is shown. Observed values (obs) were compared with controls in which PSI values in different tissues were randomly permuted (ctl). Comparisons marked with an asterisk (*) were significant by Mann-Whitney U test ($P < 0.005$).

exhibit substantially elevated KSI values relative to shuffled controls (Fig. 4D and table S7).

The above observations suggest a model in which tissue-regulated alternative splicing delimits the scope of tissues in which a kinase can phosphorylate a target. For example, the TJP1 protein mentioned above is a cytoplasmic constituent of the tight junction complex implicated in the timing of tight junction formation, and its phosphorylation is involved in tight junction dynamics (26, 27). The testes display unique tight junction biology in that tight junctions regularly dissolve and re-form to permit passage of preleptotene spermatocytes (28). The specific exclusion of exon 20 in the mammalian testis may allow TJP1 to escape phosphorylation that would otherwise alter the tight junction association kinetics required for normal testis function (29). Another example, with KSI value closer to the mean value, is an alternative exon in Droscha, a protein required for processing of microRNA primary transcripts (fig. S13). Phosphorylation of Droscha confers nuclear localization, which is required for its normal function in microRNA biogenesis (30). Therefore, splicing of Droscha may be used to alter the amount of phosphorylatable Droscha protein, potentially influencing microRNA abundance in different cells or tissues.

We have identified a large number of other exon-kinase pairs with elevated KSIs, including prominent kinases involved in development, cell cycle, and cancer (e.g., Akt1, Clk2, PKC, Src) and targets with important roles in tissue biology such as Atf2, Enah, and Vegfa (fig. S14 and table S7). Their elevated KSIs suggest that splicing is often used to focus the scope of signaling networks, connecting specific kinases to specific targets in a more cell- or tissue-restricted fashion than would occur from expression alone.

Taken together, the analyses described here reveal two disparate facets of mammalian alternative splicing. We identify a core of ~500 exons with conserved alternative splicing in mammals and high sequence conservation. These exons often encode phosphorylation sites, and their tissue-specific splicing is likely to have substantial impacts on the outputs of signaling networks. Conversely, we observe extensive variation in the splicing of these exons between species, often exceeding intraspecies differences between tissues, suggesting that changes in splicing patterns often contribute to evolutionary rewiring of signaling networks.

References and Notes

1. S. Stamm *et al.*, *Gene* **344**, 1 (2005).
2. L. F. Lareau, A. N. Brooks, D. A. Soergel, Q. Meng, S. E. Brenner, *Adv. Exp. Med. Biol.* **623**, 190 (2007).
3. E. T. Wang *et al.*, *Nature* **456**, 470 (2008).
4. Q. Pan, O. Shai, L. J. Lee, B. J. Frey, B. J. Blencowe, *Nat. Genet.* **40**, 1413 (2008).
5. D. Brawand *et al.*, *Nature* **478**, 343 (2011).
6. Materials and methods are available as supplementary materials on Science Online.

7. E. T. Chan *et al.*, *J. Biol.* **8**, 33 (2009).
8. C. Trapnell *et al.*, *Nat. Biotechnol.* **28**, 511 (2010).
9. N. Jelen, J. Ule, M. Živin, R. B. Darnell, *PLoS Genet.* **3**, 1838 (2007).
10. G. Lev-Maor *et al.*, *PLoS Genet.* **3**, e203 (2007).
11. G. W. Yeo, E. Van Nostrand, D. Holste, T. Poggio, C. B. Burge, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2850 (2005).
12. X. Xiao *et al.*, *Nat. Struct. Mol. Biol.* **16**, 1094 (2009).
13. D. Baek, P. Green, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12813 (2005).
14. A. J. Matlin, F. Clark, C. W. Smith, *Nat. Rev. Mol. Cell Biol.* **6**, 386 (2005).
15. S. C. Huelga *et al.*, *Cell Rep.* **1**, 167 (2012).
16. K. B. Cook, H. Kazan, K. Zuberi, Q. Morris, T. R. Hughes, *Nucleic Acids Res.* **39** (Database issue), D301 (2011).
17. R. Sorek, G. Ast, *Genome Res.* **13**, 1631 (2003).
18. A. N. Ladd, T. A. Cooper, *Genome Biol.* **3**, reviews0008 (2002).
19. E. T. Wang *et al.*, *Cell* **150**, 710 (2012).
20. W. Huang *et al.*, *Nat. Protoc.* **4**, 44 (2009).
21. W. Huang *et al.*, *Nucleic Acids Res.* **37**, 1 (2009).
22. I. M. Shapiro *et al.*, *PLoS Genet.* **7**, e1002218 (2011).
23. J. C. Obenauer, L. C. Cantley, M. B. Yaffe, *Nucleic Acids Res.* **31**, 3635 (2003).
24. P. V. Hornbeck, I. Chabra, J. M. Kornhauser, E. Skrzypek, B. Zhang, *Proteomics* **4**, 1551 (2004).
25. N. Dephoure *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10762 (2008).
26. G. Samak, S. Aggarwal, R. K. Rao, *Am. J. Physiol. Gastrointest. Liver Physiol.* **301**, G50 (2011).
27. E. Sabath *et al.*, *J. Cell Sci.* **121**, 814 (2008).
28. D. Mruk, C. Y. Cheng, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**, 1621 (2010).
29. S. Aggarwal, T. Suzuki, W. L. Taylor, A. Bhargava, R. K. Rao, *Biochem. J.* **433**, 51 (2011).
30. X. Tang, Y. Zhang, L. Tucker, B. Ramratnam, *Nucleic Acids Res.* **38**, 6610 (2010).
31. G. Yeo, C. B. Burge, *J. Comput. Biol.* **11**, 377 (2004).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6114/1593/DC1
Materials and Methods

Figs. S1 to S15

Tables S1 to S7

References (32–46)

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C/EBP Transcription Factors Mediate Epicardial Activation During Heart Development and Injury

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The epicardium encapsulates the heart and functions as a source of multipotent progenitor cells and paracrine factors essential for cardiac development and repair. Injury of the adult heart results in reactivation of a developmental gene program in the epicardium, but the transcriptional basis of epicardial gene expression has not been delineated. We established a mouse embryonic heart organ culture and gene expression system that facilitated the identification of epicardial enhancers activated during heart development and injury. Epicardial activation of these enhancers depends on a combinatorial transcriptional code centered on CCAAT/enhancer binding protein (C/EBP) transcription factors. Disruption of C/EBP signaling in the adult epicardium reduced injury-induced neutrophil infiltration and improved cardiac function. These findings reveal a transcriptional basis for epicardial activation and heart injury, providing a platform for enhancing cardiac regeneration.

During embryogenesis, the epicardium secretes mitogenic factors to promote cardiomyocyte proliferation and provides multipotent progenitor cells to form the coronary vasculature and the fibrous architecture of the heart (1). Cells of the adult epicardium are typically quiescent but are rapidly activated in response to cardiac injury, promoting cell cycle reentry and embryonic gene expression (2–10). Although several recent lineage-tracing experi-

ments demonstrated the presence of multipotent cardiovascular progenitor cells within the activated adult epicardium (3, 5, 8, 9), there has been a lack of functional studies that directly manipulate gene expression specifically in the adult epicardium to evaluate its contribution to cardiac regeneration and repair. Here, we report the transcriptional mechanisms underlying epicardial activation during cardiac development and repair, and a functional link between the

adult epicardium and cardiac remodeling following ischemic injury.

Identification of enhancer elements that exhibit activity in the mouse embryonic epicardium. To decipher the transcriptional basis of epicardial activation, we sought to identify cis-regulatory DNA sequences sufficient to confer epicardial expression during development and injury. We therefore designed a mouse embryonic heart organ culture and transfection assay to facilitate delivery of reporter plasmids to the epicardium (Fig. 1A) and later rapid screening of epicardial enhancer elements in luciferase reporter assays.

Epicardium development is evolutionarily conserved from fish to mammals (11, 12). In both zebrafish and mice, retinaldehyde dehydrogenase 2 (RALDH2), Wilms tumor 1 (WT1), transcription factor 21 (TCF21), and T-box 18 (TBX18) transcription factors are highly enriched in the embryonic epicardium (6, 11–14), and their expression is reactivated in the adult epicardium after injury (2, 6) (fig. S1). We reasoned that the epicardial enhancers might reside in evolutionarily conserved regions (CRs) associated with epicardial marker genes. Among the 39 CRs that encompass 740 kb of genomic regions (fig. S2 and table S1), we identified sequences *Raldh2* CR2 and *Wt1* CR14 as promising epicardial enhancer candidates, showing robust activity in the embryonic day 11.5 (E11.5) epicardium but not in human embryonic kidney 293 (HEK293) cells that lack expression of epicardial marker genes (Fig. 1, B to D, and fig. S3).

Raldh2 CR2 and *Wt1* CR14 are localized in introns 35 and 15 kb downstream of the transcriptional start site, respectively (fig. S4). We next examined whether these enhancers drive epicardial gene expression in vivo. We engineered enhancer-lacZ transgenic mice and observed robust β -galactosidase expression throughout the embryonic epicardium (Fig. 1, E and F). We also generated bacterial artificial chromosome (BAC) transgenic mice that express an enhanced green fluorescent protein (*egfp*) reporter gene in a 225-kb mouse *Raldh2* BAC or a 186-kb mouse *Wt1* BAC (fig. S5). Deletion of putative enhancer sequences resulted in a drastic loss of GFP expression in the epicardium (Fig. 1, E and F) and other tissues (fig. S6). Together, these results identify *Raldh2* CR2 and *Wt1* CR14 as enhancers that are sufficient and necessary to direct gene expression in the epicardium.

Both RALDH2 and WT1 are important regulators of epicardial functions and heart development (1, 15–17). Deletion analyses of *Raldh2* CR2 revealed a 160–base pair (bp) region that

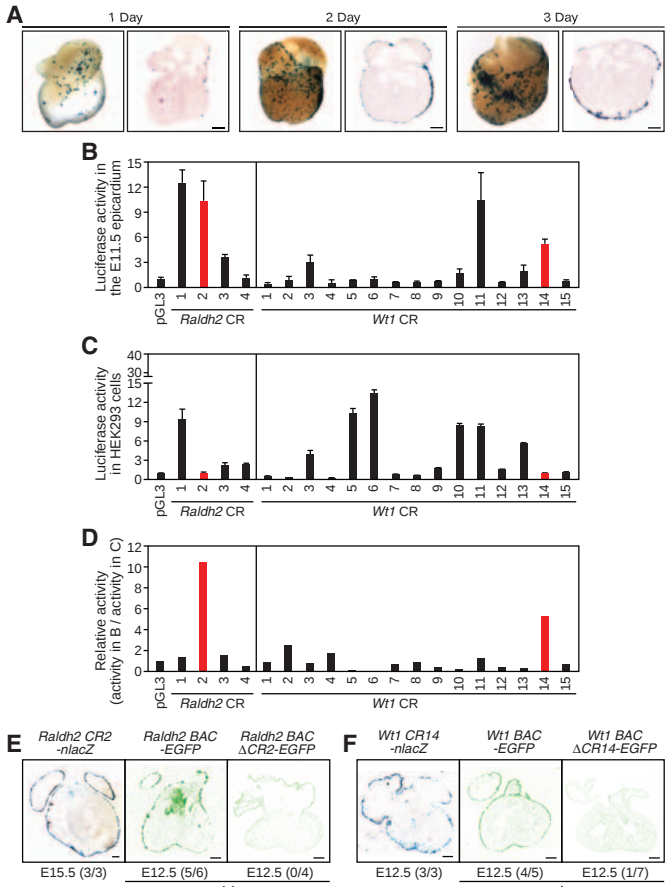
directed robust epicardial enhancer activity (Fig. 2, A and B). In two independent transgenic mouse lines that we examined, the epicardial activity of the 160-bp *Raldh2* CR2 enhancer initiated at E11.5 (18), persisted throughout gestation, and decreased postnatally (Fig. 2C and fig. S7). We observed a similar temporal pattern of epicardial activity for the 635-bp *Wt1* CR14 enhancer (Fig. 2D) (5, 6). Reporter expression was also detected in mesothelial cells and other tissues (figs. S8 and S9). Further deletion mapping of *Wt1* CR14 revealed a region of 53 bp that showed complete activity in the epicardium (Fig. 2, E and F, and fig. S9E).

Activation of epicardial enhancers by a C/EBP-dependent transcriptional combinatorial code. To search for the transcription factors that regulate the epicardial enhancers of *Raldh2* and *Wt1*, we first isolated lacZ⁺ epicardial cells from E11.5 *Tcf21*^{lacZ} hearts (19), performed a microarray analysis of gene expression, and assembled a list of epicardium-enriched transcription factors (fig. S10). Second, analyses of the 160-bp *Raldh2* CR2 and the 53-bp *Wt1* CR14 minimal enhancer sequences by transcription element search system (TESS) revealed that both enhancers contain putative C/EBP binding sites (fig. S11), and C/EBP β was enriched in epicardial cells in our microarray analysis (fig. S10).

The C/EBP family of basic leucine zipper (bZIP) transcription factors consists of six members in mammals (C/EBP α , β , δ , ϵ , γ , and ζ) (20). Quantitative analyses of C/EBP family gene expression in E11.5 *Tcf21*^{lacZ} epicardial cells revealed the expression of most members and enrichment of *Cebpb* and *Cebpd* transcripts in the embryonic epicardium (Fig. 3, A and B). Epicardial expression of C/EBP β mRNA and proteins was further confirmed (fig. S12).

We investigated whether C/EBP transcription factors bind and activate the *Raldh2* and *Wt1* enhancers. C/EBP proteins recognize a consensus sequence of (A/C)TTNCNN(A/C)A (21). The *Raldh2* CR2 and *Wt1* CR14 enhancers contain multiple putative C/EBP binding sites (fig. S11). Electrophoresis mobility shift assays (EMSAs) showed robust binding of C/EBP β to 9 (out of 10) predicted sites (Fig. 3C and fig. S13). Furthermore, overexpression of C/EBP α , C/EBP β , and C/EBP δ in HEK293 cells activated the 160-bp *Raldh2* CR2 enhancer and the 635-bp *Wt1* CR14 enhancer by two- to sixfold compared with mutant enhancers that could not bind C/EBP (Fig. 3D and fig. S14). In addition, we observed reduced expression of *Raldh2* and *Wt1* in primary epicardial cells in which *Cebpa*, *Cebpb*, or *Cebpd* were knocked down individually (fig. S15). Collectively, these data suggest that C/EBP α , C/EBP β ,

Fig. 1. Functional screen and identification of epicardial enhancers. (A) Epicardial lacZ expression in E11.5 mouse hearts 1 to 3 days after transfection of a CMV-lacZ plasmid. Whole-mount and transverse-section views are presented. (B and C) Enhancer activity of each conserved region (CR) in the epicardium [(B), $n = 2$ to 4 hearts] and in HEK293 cells [(C), $n = 3$] (mean $\pm$ SEM). (D) Relative activity. The data for *Raldh2* CR2 and *Wt1* CR14 are highlighted in red. (E and F) *Raldh2* CR2 and *Wt1* CR14 are sufficient and necessary to direct epicardial gene expression. Displayed are transgenic hearts that express a nuclear lacZ (nlacZ) driven by an enhancer (left), an EGFP reporter in a control BAC (middle), or an EGFP reporter in an enhancer-deleted BAC (right). The number of embryos that show epicardial reporter activity out of the total number of transgenic embryos is shown. Scale bars, 200 μ m. * $P < 0.05$; ** $P < 0.01$.



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and C/EBP δ may have redundant functions in regulating *Raldh2* and *Wt1* gene expression.

We next determined whether C/EBP binding is required for enhancer activity in the embryonic epicardium. Mutations of C/EBP sites resulted in a substantial reduction of enhancer activity in transgenic embryos and heart organ cultures (Fig. 3E and figs. S16 and S17). These data support the conclusion that the C/EBP binding sites are essential for the activity of the *Raldh2* CR2 and *Wt1* CR14 enhancers in the epicardium during development.

To express a dominant negative C/EBP (ACEBP) in the embryonic epicardium in vivo, we searched for another independent promoter or enhancer that exhibits epicardial activity during development. We identified *Uroplakin 3b* (*Upk3b*) as an E11.5 epicardium-enriched gene in our microarray analysis and found its expression to be highly restricted to the mesothelial cells that cover the internal organs, including the heart (fig. S18). We characterized the promoter of *Upk3b* and demonstrated that it indeed directed specific expression of a β -galactosidase reporter in mesothelial cells at E12.5 (Fig. 3F). Next, we verified that ACEBP inhibited transcriptional activity of C/EBP family members through cytoplasmic sequestration (fig. S19). We then generated transgenic mouse embryos that expressed a Flag-tagged ACEBP

under the control of the *Upk3b* promoter and examined gene expression in the epicardium. Analysis of E12.5 transgenic hearts revealed a substantial loss of RALDH2 and WT1 expression in ACEBP-expressing epicardial cells (Fig. 3G). These results suggest that C/EBP transcription factors are required for *Raldh2* and *Wt1* epicardial enhancer activity and gene expression in the embryonic epicardium.

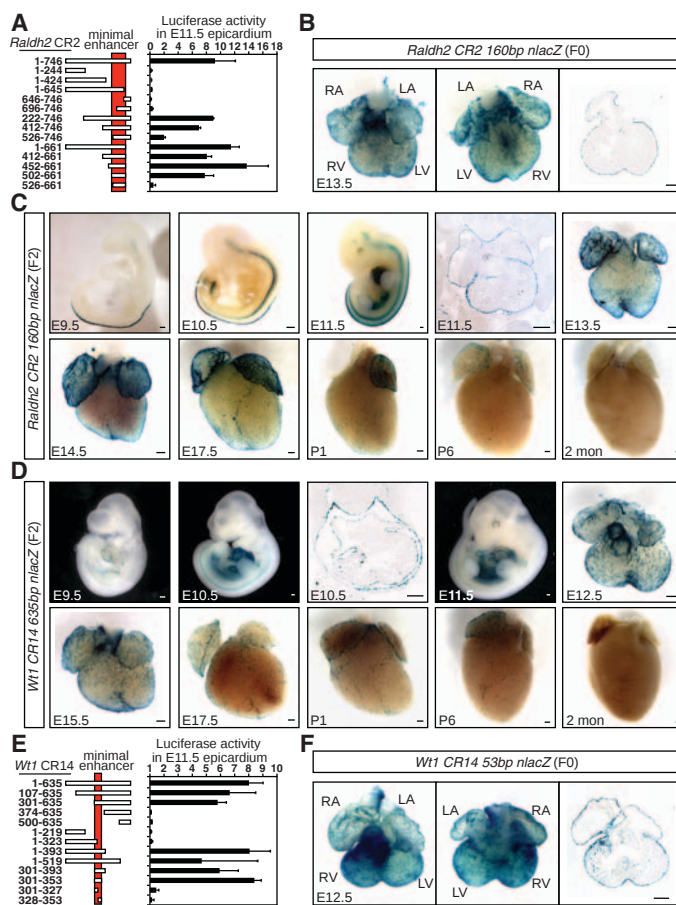
To further delineate the transcriptional code governing the *Raldh2* and *Wt1* epicardial enhancers, we generated deletion mutants with either 20- or 3-bp deletions across the minimal enhancer regions and examined their activity in ex vivo epicardial luciferase assays. Such deletion scanning analyses revealed a critical region in the *Raldh2* CR2 that contains a conserved HOX:MEIS composite binding site (Fig. 3H and fig. S20, A to C) and a region in the *Wt1* CR14 that contains a predicted binding site for the CP2 subfamily of Grainyhead (GRH) transcription factors (Fig. 3I and fig. S23, A and B). We next examined the abundance and expression pattern of MEIS, HOX, and GRH families of transcription factors and found that several members are expressed and enriched in E11.5 epicardial cells (figs. S20 to S24). In addition, we performed chromatin immunoprecipitation (ChIP) analyses in primary epicardial cells, which detected the binding of MEIS1/2, HOX2B, C/EBP α ,

and C/EBP δ to the endogenous *Raldh2* CR2 enhancer and the binding of several CP2 family and C/EBP family proteins to the *Wt1* CR14 locus (fig. S25). We further observed simultaneous binding and strong synergistic activation of the *Wt1* CR14 enhancer by C/EBP and CP2 family proteins (fig. S25). Moreover, mutant enhancers with point mutations that abolish either HOX or MEIS binding in the *Raldh2* CR2 or mutations that abolish CP2 binding in the *Wt1* CR14 region showed diminished epicardial activity in both organ cultures and transgenic embryos (Fig. 3, J to M). Altogether, our data suggest a combinatorial code of C/EBP, HOX, and MEIS in regulating the epicardial activity of the *Raldh2* CR2, and a role of C/EBP and the CP2 subfamily of Grainyhead transcription factors in governing the *Wt1* CR14 enhancer activity.

C/EBP-dependent reactivation of epicardial enhancer activity and gene expression in adult injured hearts. If reexpression of the developmental gene program in the adult epicardium after myocardial injury involves the reactivation of embryonic epicardial enhancers, the *Raldh2* CR2 and *Wt1* CR14 enhancers should be reactivated after injury. We examined our stable transgenic mouse lines that harbor a *Raldh2* or *Wt1* epicardial enhancer-driving *nlacZ* and subjected these adult animals to either permanent or transient coronary artery ligation surgery to induce myocardial infarction (MI) or ischemia reperfusion (IR), respectively. One day after MI, intense β -galactosidase activity was detected in the epicardium overlying the infarct zone and the border zone, and the expression was maintained for at least 7 days after injury (Fig. 4, A and B). Expression was most pronounced at the border zone, but much weaker and scattered expression in regions of the epicardium outside the infarct was also detected. We observed a similar pattern of activity for both enhancers after IR injury (fig. S27).

We further examined whether C/EBP transcription factors are involved in reactivation of the *Raldh2* and *Wt1* epicardial enhancers and gene expression following myocardial injury. First, we generated transgenic mice that express *lacZ* under the control of wild-type *Raldh2* and *Wt1* epicardial enhancers or mutant enhancers that lack C/EBP binding sites. After cardiac ischemic injury, *lacZ* expression was largely absent in mice with the C/EBP binding mutant enhancers (Fig. 4, C and D). Next, we examined the expression of C/EBP family members after cardiac injury. Robust up-regulation of *Cebpb* mRNA and proteins specifically in the epicardium was observed after both MI and IR injury (Fig. 4E and fig. S28). We also found strong induction of *Cebpa* and weak expression of *Cebpd* in cells of the epicardium and in the infarct area (fig. S28). Furthermore, to examine the function of C/EBP in vivo, we transduced the adult epicardium with GFP- or ACEBP-expressing adenovirus (AdGFP or AdACEBP). Viruses were carefully injected

Fig. 2. Dynamic activity of *Raldh2* and *Wt1* epicardial enhancers. (A) Epicardial enhancer activity of *Raldh2* CR2 maps to a 160-bp region (mean $\pm$ SEM, $n = 2$ to 4 hearts). (B) The *Raldh2* CR2 minimal enhancer directs epicardial expression in vivo. The staining pattern represents six out of seven F₀ transgenic embryos analyzed. (C) Temporal and spatial expression of the 160-bp *Raldh2* CR2-*nlacZ* transgene in a stable mouse line with an emphasis on the activity in the heart. (D) Dynamic activity of the 635-bp *Wt1* CR14 enhancer in a stable mouse line. (E) Mapping of the epicardial enhancer activity of *Wt1* CR14 to a 53-bp sequence ($n = 2$ to 4 hearts). (F) Epicardial expression of a 53-bp *Wt1* CR14-*nlacZ* transgenic heart, which represents three out of six transgenic embryos analyzed. LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle. Scale bars, 200 μ m.



into the pericardial space, and examination of heart cross-sections revealed restricted viral expression in the epicardium (fig. S29). In the epicardial cells infected with AdACEBP, the injury-induced expression of *RALDH2* and *WT1* was attenuated (Fig. 4F). These results support the conclusion that C/EBP proteins mediate injury-induced activation of *Raldh2* and *Wt1* epicardial enhancers and gene expression in the adult epicardium.

Inhibition of C/EBP signaling in the adult epicardium confers cardioprotection. We investigated whether expression of ACEBP in the adult epicardium could result in measurable changes in the function of ischemic hearts. Cardiac function following ischemia reperfusion was assessed by ejection fraction (EF) using magnetic resonance imaging. At both 4 and 12 weeks, cardiac function was significantly improved in the AdACEBP-injected mice compared with that of AdGFP-injected mice (Fig. 4G). The EF for AdACEBP versus AdGFP was $53.1 \pm 3.1\%$ versus $42.6 \pm 4.2\%$ at 4 weeks ($P < 0.05$) and $55.9 \pm 3.5\%$ versus $43.0 \pm 5.7\%$ at 12 weeks ($P < 0.05$). Moreover, AdACEBP-infected hearts showed a pronounced 2.5-fold reduction in the fibrotic area, compared with AdGFP-infected hearts after ischemia reperfusion (Fig. 4H). These data indicate that disruption of C/EBP activation in the epicardium of injured hearts can lead to improved contractile function and decreased myocardial fibrosis. Although at the earliest time point of analysis (1 week after surgery), the EF of AdACEBP-injected hearts ($54.6 \pm 5.0\%$) was not statistically significantly different from that of AdGFP-injected hearts ($44.7 \pm 5.1\%$, $P = 0.091$) (Fig. 4G), the ~10% improvement of EF in the AdACEBP-treated group was already present, suggesting that the key process affected by AdACEBP infection likely occurred within the first week after IR injury.

Inflammation is one of the earliest responses after tissue injury. Neutrophil influx is associated with expansion of IR-induced cardiac injury, and interventions targeting neutrophil infiltration confer cardioprotection (22, 23). Therefore, we examined neutrophil recruitment in injured hearts 1 day after IR. Intriguingly, we observed that many GR-1–positive neutrophils localized on the epicardial surface and in the subepicardial space in addition to the infarct area (Fig. 4I and fig. S30). Moreover, the neutrophil count in the AdACEBP-injected hearts was only 25% of that in the AdGFP-injected hearts (Fig. 4J). These results suggest an important function of C/EBP-mediated epicardial activation in promoting leukocyte recruitment and inflammatory responses. Notably, inactivation of C/EBP β in lung epithelial cells was also recently found to result in blunted neutrophil influx and pulmonary inflammation in response to cigarette smoke (24), suggesting a general role of C/EBP in the epithelium in regulating the innate immune response during tissue injury repair.

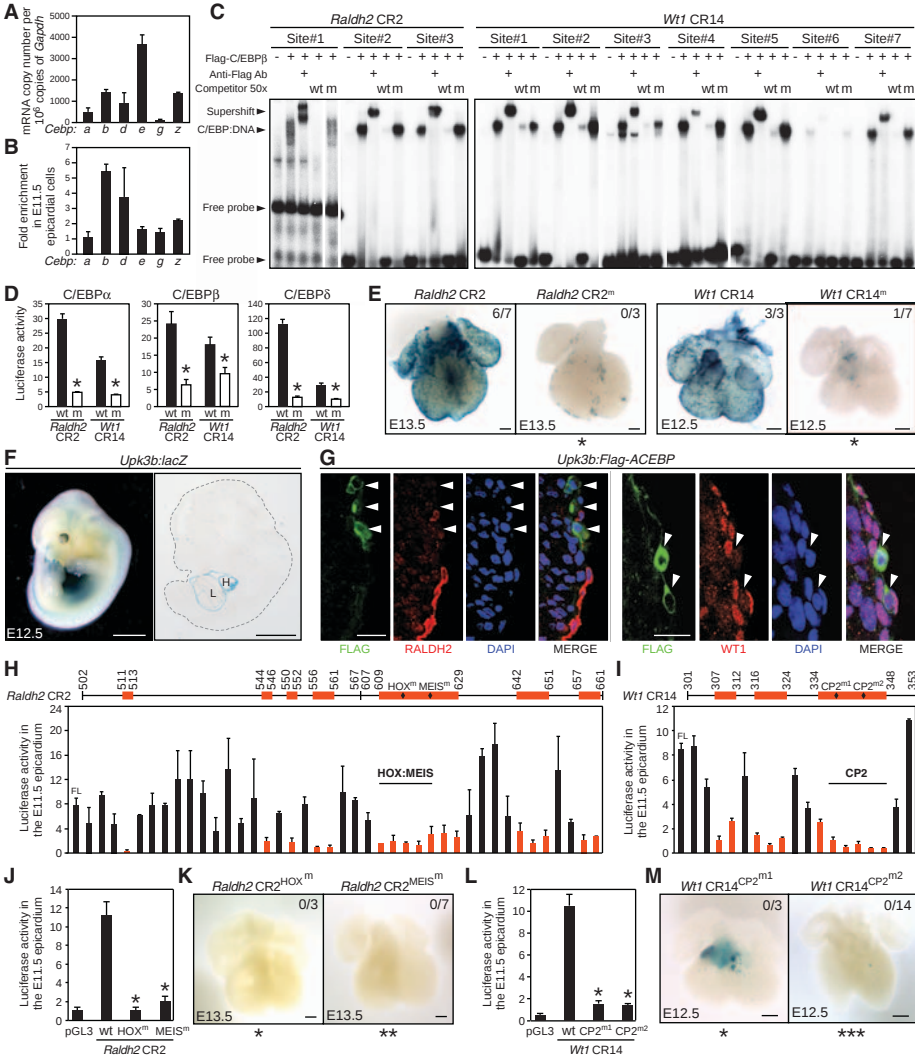


Fig. 3. A C/EBP-dependent combinatorial code for *Raldh2* and *Wt1* activation in the embryonic epicardium. (A and B) Quantitative analyses of mRNA abundance and relative enrichment of C/EBP family members in E11.5 epicardial cells ($n = 3$). (C) Binding of C/EBP β proteins to predicted sites. (D) Activation of the wild-type (wt) but not mutant (m) enhancers by C/EBP proteins in HEK293 cells ($n = 3$). (E) Mutations of C/EBP sites reduce epicardial enhancer activity in vivo. (F) X-gal stain of a transgenic embryo. H, heart; L, Liver. (G) Immunostaining of E12.5 transgenic hearts. DAPI, 4',6-diamidino-2-phenylindole. (H and I) Three-base-pair deletion scanning analyses of minimal enhancers ($n = 2$ to 6 hearts). The numbers on the top denote the positions of the critical regions (red) revealed by functional mapping. (J to M) Point mutations in either HOX, MEIS, or CP2 binding sites abolish epicardial enhancer activity in heart organ cultures [(J and L), $n = 3$ hearts] and transgenic embryos (K and M). For (E), (K), and (M), the number of epicardial lacZ⁺ embryos out of the number of transgenic embryos analyzed is presented in the upper right corner. Scale bars: 2 mm (F), 200 μ m [(E), (K), and (M)], 100 μ m (G). All error bars are SEM. In statistical analyses, all mutants were compared with wild-type controls. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

Discussion. Our work reveals a transcriptional basis of epicardial activation in both heart development and the injury response. Our data support a model (fig. S31) in which members of the C/EBP family of transcription factors are activated in the epicardium in response to both developmental cues and injury signals, and function together with HOX, MEIS, and Grainyhead transcription factors to establish a transcriptional code for embryonic gene expression in the epicardium. Grainyhead transcription factors have been impli-

cated in epithelial barrier formation and wound healing in fruit flies and mice (25, 26). Our study also suggests a potentially important role of the Grainyhead family in the injury response and function of the epithelial cells that encapsulate the heart. In addition, the present work uncovers a previously unappreciated role of the epicardium in regulation of the inflammatory response and neutrophil infiltration after injury. In this regard, manipulations that inhibit neutrophil recruitment in animals undergoing reperfusion following MI

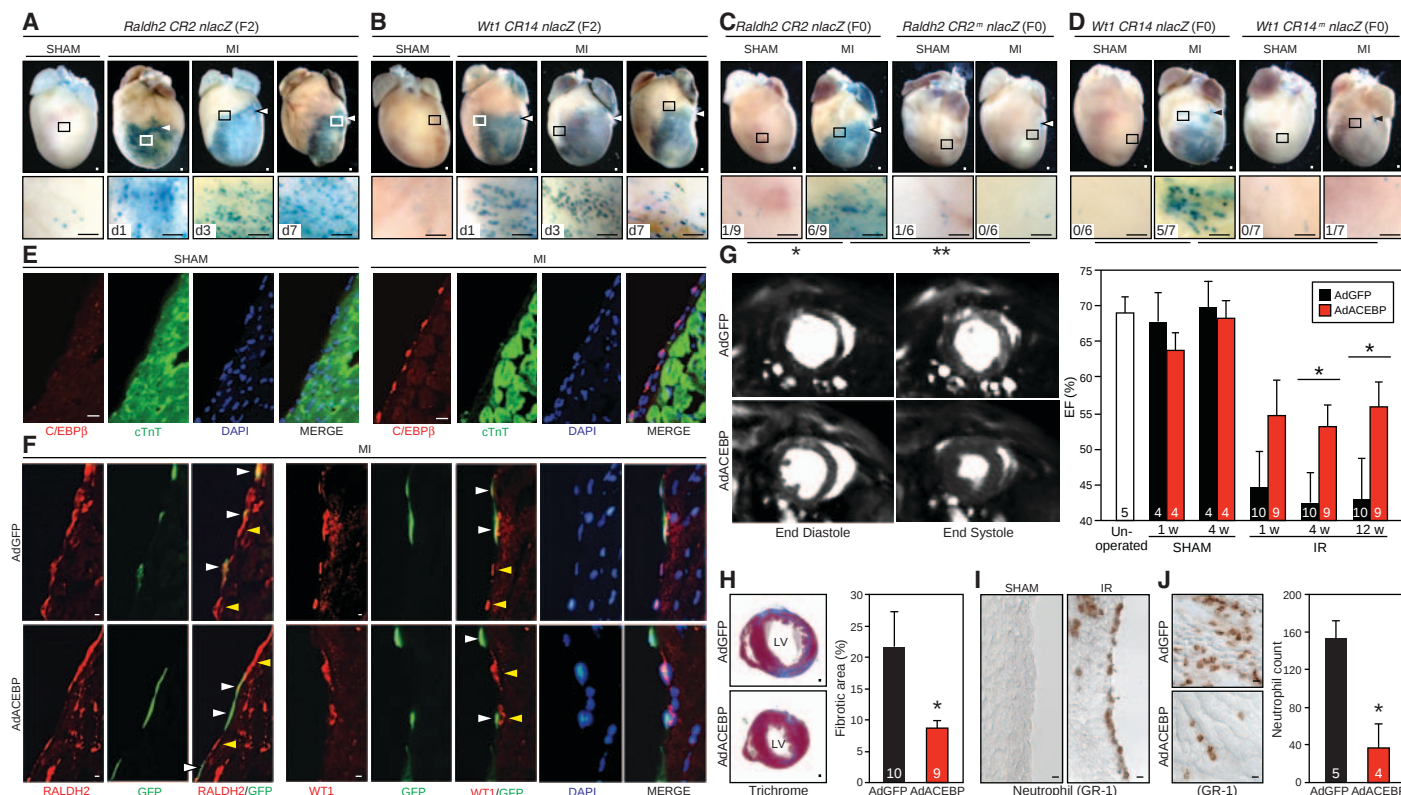


Fig. 4. Epicardial C/EBP signaling regulates injury-induced epicardial gene activation and cardiac remodeling in adult hearts. (**A** and **B**) Reactivation of *Raldh2* and *Wt1* enhancers after myocardial infarction (MI). Magnified views of boxed areas are shown below. Arrowheads mark coronary ligatures. (**C** and **D**) Diminished injury responses in transgenic mice carrying C/EBP-binding mutant enhancers. The number of epicardial lacZ⁺ hearts out of the number of transgenic hearts analyzed is shown. (**E**) Epicardial induction of C/EBP β proteins 3 days after MI. (**F**) Reduced RALDH2 and WT1 expression in AdACEBP-infected epicardial cells 3 days after MI. White arrowheads point at infected cells (GFP⁺) and yellow arrowheads mark uninfected epicardial cells (GFP⁻). (**G**) Magnetic res-

onance images (left) and ejection fraction (EF) measurements (right) of AdGFP- versus AdACEBP-injected hearts at different weeks (w) after IR surgery. (**H**) Analysis of fibrotic area (stained in blue in the trichrome stain) of heart sections from AdGFP- versus AdACEBP-injected mice 12 weeks after IR surgery. (**I**) Immunohistochemical stains of GR-1 on heart sections reveal enrichment of neutrophils around the epicardium 1 day after IR injury. (**J**) Neutrophil counts in the infarct area of AdGFP- versus AdACEBP-injected hearts 1 day after IR. LV, left ventricle. For (G), (H), and (J), the number in the column denotes the total number of animals analyzed. Scale bars, 200 μ m for (A) to (D) and (H); 20 μ m for (E), (F), (I), and (J). * P < 0.05; ** P < 0.01.

diminish infarct size (22, 23). Other inflammatory cells also contribute appreciably to cardiac damage and repair during pathological remodeling (22, 23). Future investigation of the potential role of retinoic acid (27, 28) and other epicardium-secreted cytokines and chemokines (5) as C/EBP downstream targets in regulating inflammation may uncover novel molecular targets and facilitate strategies to reduce reperfusion damage and enhance cardiac repair.

References and Notes

1. H. M. Sucov, Y. Gu, S. Thomas, P. Li, M. Pashmforoush, *Pediatr. Cardiol.* **30**, 617 (2009).
2. A. Lepilina *et al.*, *Cell* **127**, 607 (2006).
3. K. Kikuchi *et al.*, *Development* **138**, 2895 (2011).
4. K. Kikuchi *et al.*, *Dev. Cell* **20**, 397 (2011).
5. B. Zhou *et al.*, *J. Clin. Invest.* **121**, 1894 (2011).
6. F. Limana *et al.*, *J. Mol. Cell. Cardiol.* **48**, 609 (2010).
7. E. R. Porrello *et al.*, *Science* **331**, 1078 (2011).
8. N. Smart *et al.*, *Nature* **445**, 177 (2007).
9. N. Smart *et al.*, *Nature* **474**, 640 (2011).
10. E. M. Winter *et al.*, *Circulation* **116**, 917 (2007).
11. F. C. Serluca, *Dev. Biol.* **315**, 18 (2008).
12. E. M. Winter, A. C. Gittenberger-de Groot, *Cell. Mol. Life Sci.* **64**, 692 (2007).
13. B. Zhou *et al.*, *Nature* **454**, 109 (2008).
14. C. L. Cai *et al.*, *Nature* **454**, 104 (2008).
15. T. H. Chen *et al.*, *Dev. Biol.* **250**, 198 (2002).
16. A. von Gise *et al.*, *Dev. Biol.* **356**, 421 (2011).
17. O. M. Martínez-Estrada *et al.*, *Nat. Genet.* **42**, 89 (2010).
18. J. B. Moss *et al.*, *Dev. Biol.* **199**, 55 (1998).
19. J. Lu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9525 (2000).
20. H. Schrem, J. Klempnauer, J. Borlak, *Pharmacol. Rev.* **56**, 291 (2004).
21. S. Osada, H. Yamamoto, T. Nishihara, M. Imagawa, *J. Biol. Chem.* **271**, 3891 (1996).
22. L. Timmers *et al.*, *Cardiovasc. Res.* **94**, 276 (2012).
23. N. G. Frangogiannis, *Pharmacol. Res.* **58**, 88 (2008).
24. L. Didon *et al.*, *Am. J. Respir. Crit. Care Med.* **184**, 233 (2011).
25. S. B. Ting *et al.*, *Science* **308**, 411 (2005).
26. K. A. Mace, J. C. Pearson, W. McGinnis, *Science* **308**, 381 (2005).
27. C. H. Kim, *Vitam. Horm.* **86**, 83 (2011).
28. J. A. Hall, J. R. Grainger, S. P. Spencer, Y. Belkaid, *Immunity* **35**, 13 (2011).

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Supplementary Materials

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Materials and Methods
Figs. S1 to S31
Tables S1 to S3
References (29–38)

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Symmetry-Protected Topological Orders in Interacting Bosonic Systems

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Symmetry-protected topological (SPT) phases are bulk-gapped quantum phases with symmetries, which have gapless or degenerate boundary states as long as the symmetries are not broken. The SPT phases in free fermion systems, such as topological insulators, can be classified; however, it is not known what SPT phases exist in general interacting systems. We present a systematic way to construct SPT phases in interacting bosonic systems. Just as group theory allows us to construct 230 crystal structures in three-dimensional space, we use group cohomology theory to systematically construct different interacting bosonic SPT phases in any dimension and with any symmetry, leading to the discovery of bosonic topological insulators and superconductors.

For many years, the defining characteristic of a phase of matter was thought to be its symmetry, with different phases necessarily having different symmetries (1). However, through the study of high-temperature superconductors and the fractional quantum Hall (FQH) effect, it was discovered that there can be distinct quantum phases—topologically ordered phases—that cannot be distinguished by symmetry (2). A deep connection between quantum phases and quantum entanglement (3–5) indicates that topological orders are characterized by patterns of long-range entanglement (5). Recently, it was discovered that even short-range entangled states with the same symmetry can belong to different phases. These symmetric short-range entangled states are said to contain a new kind of order called symmetry-protected topological (SPT) order, (6) which is characterized by symmetry-protected gapless or degenerate edge states despite the bulk gap. Just like symmetry-breaking orders are described by group theory, we show here that SPT orders are described by group cohomology theory. This discovery expands our original understanding of possible phases in many-body systems.

A central issue is to understand what SPT phases exist. The first system known to have SPT order was the spin-1 chain with antiferromagnetic Heisenberg interactions (the so-called Haldane chains) (7, 8). This model has been generalized, leading to a complete classification of SPT orders in one-dimensional (1D) bosonic/fermionic systems (9–12). Topological insulators (13–17) with gapless edge modes protected by time-reversal symmetry and particle-number

conservation provided the first example of an SPT order in higher dimensions. The noninteracting nature of fermions in these systems allows a classification of this kind of SPT order (18, 19), whereas no SPT order exists in noninteracting bosonic systems.

However, understanding SPT orders in noninteracting systems is not sufficient, because particles in real materials do interact. In this paper, we present a systematic construction of SPT phases for interacting bosonic systems in any dimension and with any symmetry. Our construction leads to the discovery of many SPT phases in 2 and higher dimensions (see Table 1). For simplicity, we are going to first present in detail the case of the 1D Haldane chain and demonstrate the emergence of its SPT order using the group cohomology theory for time reversal symmetry. The group cohomology approach allows us to generalize the construction to higher dimensions and to all other symmetries.

The fixed-point ground-state wave function of the Haldane chain (6) takes a simple dimer form (Fig. 1), where each site contains two spin 1/2's connected into singlet pairs $|\uparrow_i^r \downarrow_{i+1}^l\rangle - |\downarrow_i^r \uparrow_{i+1}^l\rangle$ between neighboring sites (20). Time-reversal

symmetry acts as $M(T) = i\sigma_y K$ on each spin 1/2, where K is complex conjugation and σ_y is the y component of the spin operator. The wave function is invariant under the symmetry action. For each spin 1/2, $M(T)^2 = -I$, whereas on each site with two spins, $[M(T) \otimes M(T)]^2 = I$. So the states on each site form a representation of Z_2^T , the symmetry group generated by time reversal symmetry.

The wave function on a closed chain is the gapped ground state of the Hamiltonian $H = \sum_i \sigma_i^r \cdot \sigma_{i+1}^l$, with antiferromagnetic Heisenberg interactions between each pair of spin 1/2's on neighboring sites where σ_i^l and σ_i^r are spin operators for the left and right spin 1/2 on each site, respectively. The Hamiltonian is invariant under time-reversal symmetry; the ground state does not break any symmetry of the system, yet the system is far from a trivial phase, which becomes evident when we put the system on an open chain. When the chain is open, the dangling spin 1/2 at each end forms a nontrivial projective representation of Z_2^T with $M(T)^2 = -I$, which does not allow a 1D representation (21). Therefore, the degeneracy of the edge state is robust under any perturbation as long as time-reversal symmetry is preserved.

The ground-state structure giving rise to SPT order in the Haldane chain can be generalized to an arbitrary symmetry group after we relabel the spin states with group elements and express symmetry actions using group cocycles. The time-reversal symmetry group contains two elements: $Z_2^T = \{E, T\}$ with $T \circ T = E$. For the left spin 1/2 on each site, label $|\uparrow\rangle/|\downarrow\rangle$ as $|E\rangle/|T\rangle$, and for the right one, label $|\uparrow\rangle/|\downarrow\rangle$ as $|E\rangle/|-T\rangle$. The total wave function becomes

$$|\Phi\rangle = \prod_i (|T_i^r T_{i+1}^l\rangle + |E_i^r E_{i+1}^l\rangle) = \prod_i \sum_{g_i} |g_i^r = g_i, g_{i+1}^l = g_i\rangle \quad (1)$$

where $g_i \in Z_2^T$. Time-reversal symmetry then acts on the right/left spins on each site as $M(T)|E\rangle = -|T\rangle$ and $M(T)|T\rangle = |E\rangle$, which takes the form

Table 1. SPT phases in d spatial dimensions protected by some simple symmetries (represented by the symmetry groups). Z_1 means that our construction only gives rise to the trivial phase. Z_n^m means that the constructed nontrivial SPT phases plus the trivial phase are labeled by m elements in Z_n . Z means that the constructed nontrivial SPT phases are labeled by nonzero integers, whereas the trivial one is labeled by 0. Z_2^T represents time-reversal symmetry, $U(1)$ represents boson number—conservation symmetry, $SO(3)$ represents rotation symmetry, Z_n represents cyclic symmetry of order n , and D_2 represents the Klein four-group symmetry. The first row corresponds to bosonic topological insulators and the second row to bosonic topological superconductors.

Symmetry	$d = 0$	$d = 1$	$d = 2$	$d = 3$
$U(1) \rtimes Z_2^T$	Z	Z_2	Z_2	Z_2^2
Z_2^T	Z_1	Z_2	Z_1	Z_2
$U(1)$	Z	Z_1	Z	Z_1
$SO(3)$	Z_1	Z_2	Z	Z_1
$SO(3) \times Z_2^T$	Z_1	Z_2^2	Z_2	Z_2^3
Z_n	Z_n	Z_1	Z_n	Z_1
$Z_2^T \times D_2 = D_{2h}$	Z_2^2	Z_2^4	Z_2^6	Z_2^9

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$$M^r(g)|g_0^r\rangle = v_2^{s(g)}(g_0^r, g^{-1}g^*, g^*)|gg_0^r\rangle, g^* = E$$

$$M^l(g)|g_0^l\rangle = v_2^{-s(g)}(g_0^l, g^{-1}g^*, g^*)|gg_0^l\rangle, g^* = E$$

$$(2)$$

where for $g \in Z_2^T$, $v_2(E, T, E) = v_2(T, E, T) = -1$, and $v_2(g_0, g_1, g_2) = 1$ otherwise. $s(g) = 1$ if g is unitary and $s(g) = -1$ if g is antiunitary. Here, $v_2(g^0, g^1, g^2)$ is the nontrivial 2-cocycle of Z_2^T , which is a function from three group elements to a $U(1)$ phase factor satisfying (21)

$$v_2^{s(g)}(g_0, g_1, g_2) = v_2(gg_0, gg_1, gg_2), g \in G$$

$$(3)$$

and

$$\frac{v_2(g_1, g_2, g_3)v_2(g_0, g_1, g_3)}{v_2(g_0, g_2, g_3)v_2(g_0, g_1, g_2)} = 1$$

$$(4)$$

For an arbitrary symmetry group G , if the ground-state wave function takes the dimer form as in Eq. 1 and symmetry acts on each right/left spin as in Eq. 2, then the edge spin forms a projective representation of symmetry G (labeled by v_2), and the state contains an SPT order protected by the symmetry (9, 12).

We can also use path integrals in (1+1)D to describe the 1D SPT phases, which allow us to generalize our result to higher dimensions. Because a 1D SPT phase is described by a cocycle v_2 , we can use the very same v_2 to construct the path integral for the SPT phase. To do so, we discretize the (1+1)D space time with a branched triangulation (Fig. 2A). For the Haldane chain, we associate a $g_i \in Z_2^T$ with each vertex of the space-time complex. Time reversal acts as complex conjugation K together with a mapping from g^i to

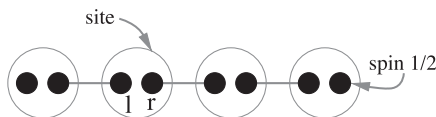


Fig. 1. Dimer form of the ground-state wave function in Haldane chain. Each site (big oval) contains two spin 1/2's (small dot), which are connected into singlet pairs (connected dots) between neighboring sites.

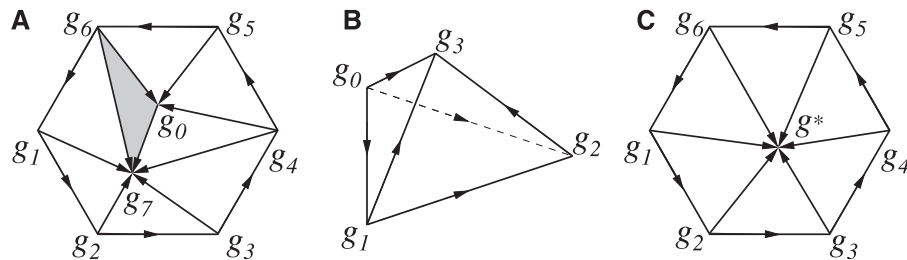


Fig. 2. (A) A branched triangulation of space time (21). Note that $s_{607} = -1$. (B) A tetrahedron, the simplest discrete closed surface. $\prod v^{s_{ijk}}(g_i, g_j, g_k) = 1$ on a tetrahedron is guaranteed by Eq. 4. Note that $s_{123} = s_{013} = 1$ and $s_{023} = s_{012} = -1$. (C) Discretized space-time manifold M_{ext} on an open disk with boundary manifold M . $g_j \in M$, g^* is in the interior of M_{ext} .

Tg^i . The path integral for the SPT phase then has the form

$$Z = |G|^{-N_v} \sum_{\{g_i\}} e^{-s(\{g_i\})},$$

$$e^{-s(\{g_i\})} = \prod_{\{ijk\}} v_2^{s_{ijk}}(g_i, g_j, g_k)$$

$$(5)$$

$|G|$ is the number of elements in G ($|G| = 2$ for Z_2^T), N_v is the number of vertices in the complex; $s_{ijk} = \pm 1$ depending on the orientation of the triangle. Because $v_2(Tg_0, Tg_1, Tg_2) = v_2^{-1}(g_0, g_1, g_2)$ (Eq. 3), the path integral is invariant under time reversal. (Similar construction works for any group.)

Because of Eq. 4, the path integral Eq. 5 actually describes a fixed-point theory, which does not change under coarse graining and retriangulation (21). For example, the path integral on the two triangulations (Fig. 2, A and C) is the same if we fix g_i 's on the boundary. Using this property, we can show that the action amplitude is always 1 on any orientable closed space-time surface, including the simplest discrete closed surface—a tetrahedron (Fig. 2B). So, g_i fluctuate strongly and the path integral describes a disordered phase that does not break the symmetry G .

To show that this path integral describes the SPT order in the Haldane chain, we need to calculate the ground-state wave function from the path integral that describes the imaginary time evolution from time $-\infty$ until time 0. In our formulation, this is equivalent to an imaginary time path integral on a space-time geometry with a boundary (at time 0). Denote the boundary as M and the whole manifold (a disk) as M_{ext} (Fig. 2A). As we are considering a fixed-point path integral, it does not matter how big the interior of M_{ext} is, and we can reduce it, for example, to just one point (Fig. 2C).

To obtain the ground-state wave function, we fix the degrees of freedom $\{g_i\}_M$ on M and find

$$\Psi(\{g_i\}_M) \propto \sum_{g^*} \prod_i v_2(g_i, g_{i+1}, g^*)$$

$$\propto \prod_i v_2(g_i, g_{i+1}, g^* = E),$$

$$(6)$$

where $\prod_i$ is the product over all triangles on M_{ext} and, for simplicity of notation, we have chosen all triangles to be oriented clockwise.

The wave function on M does not depend on the choice of g^* . Time reversal acts as complex conjugation K together with a change of basis $|E\rangle \rightarrow |T\rangle$, $|T\rangle \rightarrow |E\rangle$ on each g_i , and the wave function is invariant under this action.

To show that the wave function Eq. 6 corresponds to the dimer state Eq. 1 (Fig. 1), we first expand each g_i into two degrees of freedom h_i^r and h_{i+1}^l such that $h_i^r = h_{i+1}^l = g_i$ (Fig. 3) and the amplitude of each configuration in the wave function remains unchanged, $\Psi(\{h_i^r = h_{i+1}^l = g_i\}) = \prod_i v_2(g_i, g_{i+1}, g^*)$. We then combine h_i^r and h_{i+1}^l into one site and apply a change of basis on each site

$$|h_i^r, h_{i+1}^l\rangle' = v_2(h_i^r, h_{i+1}^l, g^*)|h_i^r, h_{i+1}^l\rangle$$

$$= v_2(g_{i-1}, g_i, g^*)|h_i^r, h_{i+1}^l\rangle$$

The amplitude of all configurations in the new basis becomes 1, $\Psi(\{h_i^r = h_{i+1}^l = g_i\}) = 1$, which can be equivalently written as a product of dimers between neighboring sites $\Psi' = \prod_i \sum_{g_i} |h_i^r = g_i, h_{i+1}^l = g_i\rangle$. In this way, we have mapped each degree of freedom g_i into a dimer and the total wave function takes the same form as Eq. 1. Moreover, time-reversal symmetry acts on the edge degree of freedom as given by Eq. 2 (20). Therefore, our path integral Eq. 5 provides a proper description of the SPT order in the Haldane chain. To generalize this path-integral formulation to all spatial dimensions d and all symmetry groups G , we note that the two cocycles $v_2(g_0, g_1, g_2)$ used in the construction have higher dimensional analogs: the $(d+1)$ cocycles $v_{d+1}(g_0, \dots, g_{d+1})$, which are maps from $d+2$ group elements to a $U(1)$ phase factor and satisfy

$$v_{d+1}^{s(g)}(g_0, g_1, \dots, g_{d+1}) =$$

$$v_{d+1}(gg_0, gg_1, \dots, gg_{d+1}), g \in G$$

and

$$\prod_{i=0}^{d+2} v_{d+1}^{(-1)^i}(g_0, \dots, g_{i-1}, g_{i+1}, \dots, g_{d+2}) = 1$$

We use each $(d+1)$ cocycle v_{d+1} to construct a fixed-point path integral to describe an SPT state in d dimensions. The path integral is constructed by (i) discretizing the $(d+1)D$ space time with triangulation [triangle in (1+1)D, tetrahedron in (2+1)D, etc.]; (ii) assigning group element-labeled degrees of freedom to the vertices; and (iii) assigning action amplitude to each

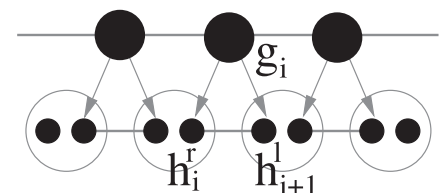


Fig. 3. Duality transformation between wave functions in Eq. 1 and Eq. 6.

simplex with the corresponding cocycle. The path integral then takes the form

$$z = |G|^{-N_v} \sum_{\{g_i\}} \prod_{\{ij...k\}} v_{d+1}^{s_{ij...k}}(g_i, g_j, \dots, g_k) \quad (7)$$

where $s_{ij...k} = \pm 1$ depends on the orientation of the simplex $ij...k$. Similar to the $(1+1)D$ case, it can be shown that the path integral is symmetric under symmetries in group G , and the action amplitude is in a fixed-point form and is quantized to 1 on a closed manifold (21). The ground-state wave function can be obtained from the action amplitude on an open geometry as discussed before $\Psi(\{g_i\}_M) = \prod_{\{i...j^*\}} v_{d+1}(g_i, \dots, g_j, g^*)$, where $\{g_i\}_M$ is on M and g^* is inside M_{ext} . $\prod_{\{i...j^*\}}$ is the product over all simplices. An exactly soluble Hamiltonian can be constructed to realize this state as the gapped ground state (21).

The nontrivial SPT order of the system can be seen explicitly from its boundary. The path integral of the degrees of freedom on the boundary can be obtained by putting the path integral on an open geometry as shown in Fig. 2C for $(1+1)D$. The manifold M now corresponds to the space-time manifold of the boundary degrees of freedom. The path integral for the boundary then reads

$$Z_b = |G|^{-N_v} \sum_{\{g_i\}} \prod_{\{i...j^*\}} v_{d+1}^{s_{i...j^*}}(g_i, \dots, g_j, g^*) \quad (8)$$

which only depends on $\{g_i\}_M$ on the boundary M and does not depend on g^* , which is inside M_{ext} .

This term can be thought of as a discretized version of the Wess-Zumino-Witten (WZW) term (22, 23) in nonlinear σ models because (i) it is a path integral of $(d-1)+1$ dimensional systems written on an extended $(d+1)D$ manifold with a boundary; (ii) the action amplitude does not depend on how the extended field in the interior of the $(d+1)D$ manifold is chosen; and (iii) its field takes value in a group G , and the path integral is invariant under the action of $g \in G$. On the other hand, this term is more general than the original continuous WZW term because it applies to discrete groups like Z_2^T while the continuous WZW term only works for continuous groups. We expect that the boundary states described by such a discretized WZW term will be gapless/degenerate as long as symmetry is not broken, similar to systems described by continuous WZW terms. This has been firmly established in $(1+1)D$ and $(2+1)D$. In $(1+1)D$, as with the example of Haldane chain, symmetry action on the edge degree of freedom does not have a 1D representation; therefore, the edge state will always be degenerate. In $(2+1)D$, it has been proven using the tool of matrix product unitary operator that the boundary must be gapless as long as symmetry is not broken (24, 25). Therefore, the boundary of the systems we constructed carries gapless/degenerate states protected by certain symmetry, which reflects the nontrivial SPT order of the system.

The numbers of nontrivial SPT phases constructed using cocycles for some simple symmetry groups are summarized in Table 1. We find one kind of bosonic topological insulator in 2D and three kinds in 3D with boson number-conservation symmetry $U(1)$ and time-reversal symmetry Z_2^T . If boson numbers are not conserved but time-reversal symmetry Z_2^T is preserved, then we find one kind of bosonic topological superconductor in every odd spatial dimension. Our construction is nonperturbative and works for strongly interacting bosonic systems. Therefore, it contributes to a more complete understanding of the topological phase diagram in strongly correlated quantum systems.

References and Notes

1. L. D. Landau, E. M. Lifschitz, *Statistical Physics: Course of Theoretical Physics Vol 5* (Pergamon, London, 1958).
2. X. G. Wen, *Int. J. Mod. Phys. B* **4**, 239 (1990).
3. A. Kitaev, J. Preskill, *Phys. Rev. Lett.* **96**, 110404 (2006).
4. M. Levin, X.-G. Wen, *Phys. Rev. Lett.* **96**, 110405 (2006).
5. X. Chen, Z.-C. Gu, X.-G. Wen, *Phys. Rev. B* **82**, 155138 (2010).
6. Z.-C. Gu, X.-G. Wen, *Phys. Rev. B* **80**, 155131 (2009).
7. F. D. M. Haldane, *Phys. Lett. A* **93**, 464 (1983).
8. I. Affleck, T. Kennedy, E. H. Lieb, H. Tasaki, *Commun. Math. Phys.* **115**, 477 (1988).
9. X. Chen, Z.-C. Gu, X.-G. Wen, *Phys. Rev. B* **83**, 035107 (2011).
10. L. Fidkowski, A. Kitaev, *Phys. Rev. B* **83**, 075103 (2011).
11. A. M. Turner, F. Pollmann, E. Berg, *Phys. Rev. B* **83**, 075102 (2011).

12. N. Schuch, D. Perez-Garcia, I. Cirac, *Phys. Rev. B* **84**, 165139 (2011).
13. C. L. Kane, E. J. Mele, *Phys. Rev. Lett.* **95**, 226801 (2005).
14. B. A. Bernevig, S.-C. Zhang, *Phys. Rev. Lett.* **96**, 106802 (2006).
15. C. L. Kane, E. J. Mele, *Phys. Rev. Lett.* **95**, 146802 (2005).
16. J. E. Moore, L. Balents, *Phys. Rev. B* **75**, 121306 (2007).
17. L. Fu, C. L. Kane, E. J. Mele, *Phys. Rev. Lett.* **98**, 106803 (2007).
18. A. Kitaev, *AIP Conf. Proc.* **1134**, 22 (2009).
19. S. Ryu, A. P. Schnyder, A. Furusaki, A. W. W. Ludwig, *New J. Phys.* **12**, 065010 (2010).
20. The usual spin 1 degree of freedom in the Haldane chain can be obtained by projecting the two spin 1/2's on each site to their symmetric subspace. Without projection, the wave function is in simpler form and still contains the same topological features.
21. See supplementary materials for details.
22. J. Wess, B. Zumino, *Phys. Lett. B* **37**, 95 (1971).
23. E. Witten, *Nucl. Phys. B* **223**, 422 (1983).
24. X. Chen, Z.-X. Liu, X.-G. Wen, *Phys. Rev. B* **84**, 235141 (2011).
25. M. Levin, Z. C. Gu, *Phys. Rev. B* **86**, 115109 (2012).

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Supplementary Materials

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Materials and Methods
Figs. S1 to S9
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Sign-Problem-Free Quantum Monte Carlo of the Onset of Antiferromagnetism in Metals

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The quantum theory of antiferromagnetism in metals is necessary for our understanding of numerous intermetallic compounds of widespread interest. In these systems, a quantum critical point emerges as external parameters (such as chemical doping) are varied. Because of the strong coupling nature of this critical point and the “sign problem” plaguing numerical quantum Monte Carlo (QMC) methods, its theoretical understanding is still incomplete. Here, we show that the universal low-energy theory for the onset of antiferromagnetism in a metal can be realized in lattice models, which are free from the sign problem and hence can be simulated efficiently with QMC. Our simulations show Fermi surface reconstruction and unconventional spin-singlet superconductivity across the critical point.

The presence of an antiferromagnetic transition in a metal is common to compounds such as electron-doped cuprates (1), iron-

based superconductors (2), and heavy fermion Kondo lattice systems (3). Whereas our understanding of quantum antiferromagnetism in insulators has seen major advances (4), analogous problems in metals are far more complicated because of the subtle interplay between the low-energy fermionic quasiparticles on the Fermi surface, and the quantum fluctuations of the antiferromagnetic order parameter. In addition, the presence of the Fermi surface has hampered large-scale numerical studies, because quantum Monte Carlo (QMC) algorithms are afflicted by the well-known fermion

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sign problem. Such algorithms express the partition function as a sum over Feynman histories, and the sign problem arises when the weights assigned to the trajectories are not all positive because of quantum interference effects. A general solution to the fermion sign problem has been proved to be in the computational complexity class of nondeterministic polynomial (NP) hard (5), and so there has been little hope that the antiferromagnetic quantum critical point could be elucidated by computational studies.

Application of the methods of quantum field theory and the renormalization group to the onset of antiferromagnetism in a metal (6) has identified (7, 8) a universal quantum field theory that captures all the singular low-energy quantum fluctuations that control the quantum critical point and deviations from the Fermi liquid physics of traditional metals. The field theory is expressed in terms of fermionic excitations in the vicinity of a finite number of “hot spots” on the Fermi surface, and is thus independent of the details of the fermionic band structure, except for the number of hot spots and Fermi velocities at the hot spots (9). Recent work (10, 11) has shown

that the renormalization group and Feynman graph expansions of the field theory flow to strong coupling in two spatial dimensions, making further analytical progress difficult.

Here, we show that the universal quantum field theory can be realized in lattice models that are free of the sign problem and so is amenable to large-scale QMC studies. Our claim does not contradict the no-go theorem of (5), because we do not provide a general recipe for eliminating the sign problem. However, we will eliminate it for the specific case of the onset of antiferromagnetic order in a two-dimensional metal, provided the perturbative arguments on the importance of the hot spots to the quantum field theory (7, 8, 10, 11) apply. Our modified lattice model has at least two bands. Therefore, in cases in which there is only a single active band at the transition, such as in the electron-doped cuprates, our method requires modifying the Fermi surface far away from the hot spots; we show that this can be done while preserving the universal low-energy properties of the antiferromagnetic critical point. On the other hand, our method applies to multiband situations (such as in the

iron-based superconductors) without changes to their Fermi surface configuration. Being a low-energy effective theory, the method will not apply where the proximity of a Mott insulator is important, as is likely the case in the hole-doped cuprates (12–16).

To illustrate our method, we consider the onset of antiferromagnetic order in a simple one-band model on the square lattice, as is appropriate for the electron-doped cuprates. The electrons, $c_{\mathbf{k}}$ (the spin index is left implicit), with dispersion $\epsilon_{\mathbf{k}}$, have a single “large” Fermi surface (Fig. 1A). The antiferromagnetic order parameter is $\vec{\phi}_{\mathbf{q}}$; we will assume the important fluctuations of $\vec{\phi}_{\mathbf{q}}$ are restricted to small values of $|\mathbf{q}|$, much smaller than the size of the Brillouin zone. The antiferromagnetic ordering wavevector is $\mathbf{K} = (\pi, \pi)$, and $\vec{\phi}_{\mathbf{q}}$ represents the electron spin density at the wavevector $\mathbf{K} + \mathbf{q}$; we will also refer to the antiferromagnetic order as spin density wave (SDW) order. We can thus write the electron part of the Hamiltonian as

$$H = \sum_{\mathbf{k}} \epsilon_{\mathbf{k}} c_{\mathbf{k}}^{\dagger} c_{\mathbf{k}} + \lambda \sum_{\mathbf{k}, \mathbf{q}} c_{\mathbf{k}+\mathbf{K}+\mathbf{q}}^{\dagger} (\vec{s} \cdot \vec{\phi}_{\mathbf{q}}) c_{\mathbf{k}} \quad (1)$$

where λ is the “Yukawa” coupling between the electrons and the SDW order, and $\vec{s}$ are the Pauli matrices. The Yukawa term is the simplest coupling consistent with translational symmetry and spin-rotation invariance, and can be derived, e.g., by decoupling of the repulsive interaction in a Hubbard model by an auxiliary field that maps to $\vec{\phi}$ in the long-wavelength limit (17). The hot spots are at $\mathbf{k}$ for which $\epsilon_{\mathbf{k}} = \epsilon_{\mathbf{k}+\mathbf{K}} = 0$ (Fig. 1A); at these points, $\vec{\phi}_{\mathbf{q}=0}$ scatters electrons between initial and final states, which are both on the Fermi surface. To obtain the electron Fermi surface in a metal with SDW order, we replace $\vec{\phi}_{\mathbf{q}}$ by its expectation value $\langle \vec{\phi}_{\mathbf{q}} \rangle = \vec{N} \delta_{\mathbf{q},0}$ (where $\vec{N}$ is the staggered magnetization) and recompute the electron dispersion; this leads to the Fermi surface reconstruction shown in Fig. 1B.

We now describe our method to deform the model, such that the sign problem is avoided, while preserving the structure of the hot spots. Let us separate the hot spots into two groups, so that $\mathbf{K}$ only connects hot spots from one group to the other. Now deform the one-band electronic dispersion to a two-band model with an additional “orbital” label so that all the hot spots in one group are on the Fermi surfaces of the first band, while the hot spots of the other group reside on the Fermi surfaces of the second band (an example of such a dispersion is shown in Fig. 1C, in which the “horizontal” and “vertical” Fermi surfaces are part of two separate electronic bands). As can be seen, the vicinities of the hot spots in the two-band model are essentially identical to those in the one-band model in Fig. 1A, and so the same low-energy theory for the onset of antiferromagnetism applies to both models. With no further assumptions, the deformed model has only positive weights in a suitable QMC realization.

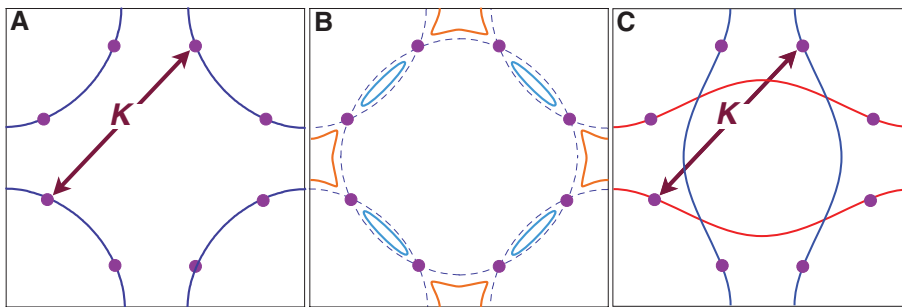


Fig. 1. (A) Fermi surface of the Fermi liquid phase of a single band model on the square lattice with unit lattice spacing. The “hot spots” are denoted by the filled circles. (B) The reconstructed Fermi surface in the metal with SDW order. The dashed lines show the Fermi surface in the metal without SDW order, and its translation by $\mathbf{K}$. Gaps have opened at the hot spots, leading to small “pocket” Fermi surfaces. (C) A deformed Fermi surface of the metal without SDW order, in which the vicinities of the hot spots are unchanged from (A). The horizontal and vertical Fermi surfaces now belong to separate electronic bands.

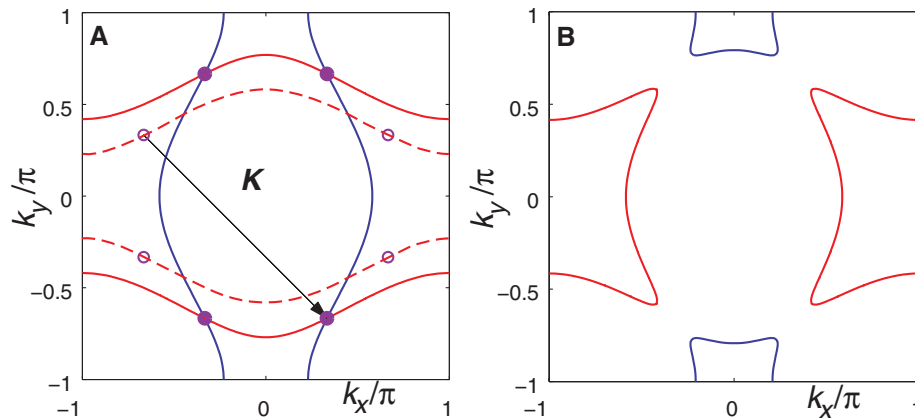


Fig. 2. (A) Fermi surfaces (solid lines) of L_F for free $\psi_{x,y}$ fermions with the parameters listed in the text. The dashed lines show the portion of the Fermi surface in Fig. 1C that was shifted by $\mathbf{K}$ to obtain the ψ_y Fermi surface. The hot spots are now at the intersections of the Fermi surfaces. (B) Mean-field $\psi_{x,y}$ Fermi surfaces with SDW order $|\langle \phi \rangle| = 0.25$.

We will write down a specific lattice model for which we will establish a sign-free Monte Carlo algorithm and then present numerical results. We begin with the band structure of the $c_{\mathbf{k}}$ electrons in Fig. 1C. We write the band with vertical Fermi surfaces in terms of fermions ψ_x with $c_{\mathbf{k}} \rightarrow \psi_{x,\mathbf{k}}$, and the band with horizontal Fermi surfaces in terms of fermions ψ_y with $c_{\mathbf{k}} \rightarrow \psi_{y,\mathbf{k}+\mathbf{K}}$. This leads to the $\psi_{x,y}$ Fermi surfaces shown in Fig. 2A. Then our model has the action $S = S_F + S_\phi = \int_0^\beta d\tau (L_F + L_\phi)$ with

$$L_F = \sum_{i,j,a=x,y} \psi_{ai}^\dagger [(\partial_\tau - \mu)\delta_{ij} - t_{a,ij}] \psi_{aj} + \lambda \sum_i \psi_{xi}^\dagger (\vec{s} \cdot \vec{\phi}_i) \psi_{yi} + H.c.$$

$$L_\phi = \frac{1}{2} \sum_i \frac{1}{c^2} \left(\frac{d\vec{\phi}_i}{d\tau} \right)^2 + \frac{1}{2} \sum_{\langle ij \rangle} (\vec{\phi}_i - \vec{\phi}_j)^2 + \sum_i \left(\frac{r}{2} \vec{\phi}_i^2 + \frac{u}{4} (\vec{\phi}_i^2)^2 \right) \quad (2)$$

Here i, j run over the sites of the square lattice, τ is the imaginary time, and β is the inverse tem-

perature. The parameter r will be used to tune across the quantum critical point, and u is a nonlinear self-coupling of $\vec{\phi}$. The $\psi_x(\psi_y)$ fermion hops along the horizontal (vertical) direction with an amplitude $t_{\parallel} = -1$ (+1), and along the vertical (horizontal) direction with an amplitude $t_{\perp} = -0.5$ (0.5), respectively; the resulting band structure is shown in Fig. 2A (solid lines). The model has C_4 symmetry, and its apparent violation is an artifact of the shifting of the ψ_y fermions by $\mathbf{K}$. We chose the chemical potential $\mu_1 = \mu_2 = -0.5$, $c = 1$, $u = 1$, and $\lambda = 1$.

By construction, the modified two-band model has the same hot spot structure as the original one-band model. Therefore, we argue that it preserves the universal properties of the antiferromagnetic transition. We prove (9) that the introduction of the second band eliminates the sign problem in this model.

It is possible to analytically integrate out $\vec{\phi}$ in Eq. 2 and establish equivalence to a large class of Hubbard-like models to which our method applies. However, we choose to keep $\vec{\phi}$ as an independent degree of freedom because it keeps the physics transparent and streamlines the analysis.

We have performed determinant Monte Carlo simulations of the action (2) using the algorithm described in (18–20), for systems of linear size up to $L = 14$ and inverse temperature $\beta = 14$, with either periodic or antiperiodic boundary conditions. An imaginary time step of $\Delta\tau = 0.1$ was used in most of the calculations; we checked that the results do not change for $\Delta\tau = 0.05$. Up to 50,000 Monte Carlo sweeps were performed for each run, giving a statistical error for most measured quantities of a few percent.

First, we present results showing the reconstruction of the Fermi surface across the SDW transition. Figure 3 shows the fermion occupation number summed over the two flavors of fermions as a function of quasi-momentum. The Fermi surfaces are clearly visible as discontinuities. $r = 0.5$ is found to be on the disordered side of the SDW critical point, and the Fermi surface closely resembles the one in Fig. 2A. At $r = 0$, a gap opens at the hot spots, and the Fermi surface is reconstructed into electron and hole pockets, as in the SDW ordered state in Fig. 2B. Decreasing r further to -0.5 increases the magnitude of the SDW order parameter and causes the hole pockets to disappear and the electron pockets to shrink.

To examine the magnetic transition, we computed the SDW susceptibility $\chi_\phi = \sum_{i,j} \int_0^\beta d\tau \langle \vec{\phi}_i(\tau) \cdot \vec{\phi}_j(0) \rangle$. Figure 4A shows χ_ϕ normalized by $L^2\beta$ as a function of r . To extract information about the zero-temperature limit, we scale β with the linear system size; in the appropriate units, $\beta = L$ was used. We observe a rapid upturn in χ_ϕ near $r = 0.25$. For $r < 0.25$, $\chi_\phi/(L^2\beta)$ for different system sizes and inverse temperatures nearly collapse on top of each other, which is the expected behavior on the ordered side of the transition. The results are consistent with a second-order transition at $r_c \approx 0.25$. This is further supported by the Binder cumulant in Fig. 4B, where we observe the expected behavior in both phases, separated by a critical point at $r_c = 0.25 \pm 0.1$.

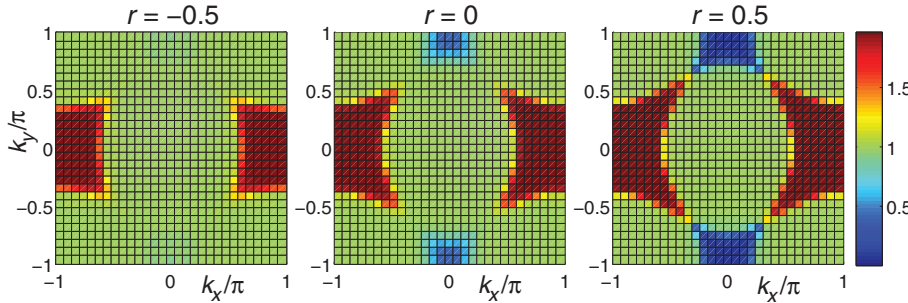


Fig. 3. Quantum Monte-Carlo results for the fermion occupation number $\eta_{\mathbf{k}} = \langle \psi_{x\mathbf{k}}^\dagger \psi_{x\mathbf{k}} + \psi_{y\mathbf{k}}^\dagger \psi_{y\mathbf{k}} \rangle / 2$ as a function of $\mathbf{k}$ across the Brillouin zone, for systems with $L = 14$, $\beta = 14$, and $r = -0.5, 0, 0.5$. To enhance the resolution, we combined results from simulations with either periodic or antiperiodic boundary conditions in the x and y directions. Despite appearances, full square-lattice symmetry is preserved in all our computations for the original $c_{\mathbf{k}}$ fermions.

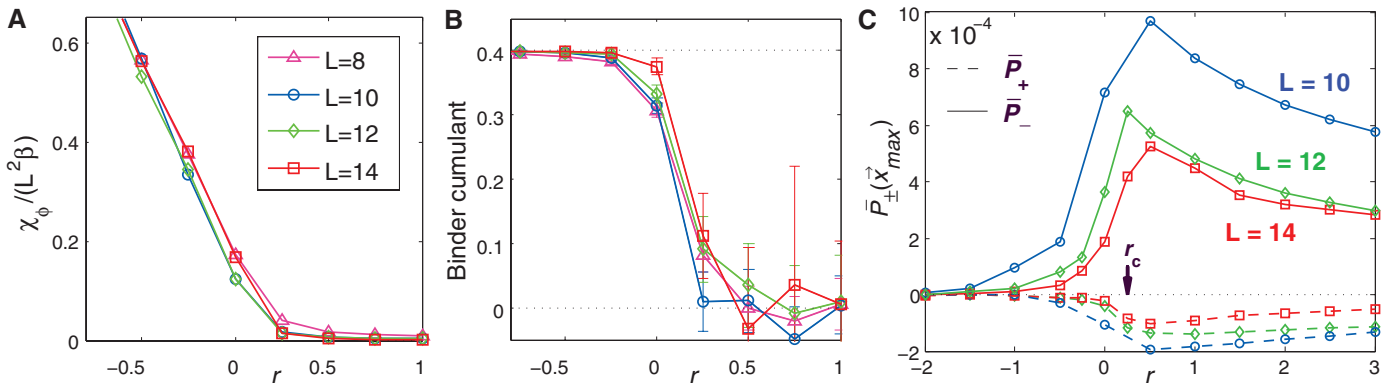


Fig. 4. (A) The SDW susceptibility χ_ϕ , normalized by $L^2\beta$, as a function of r , for systems of size $L = 8, 10, 12, 14$ and $\beta = L$ for each curve. The statistical errors in χ_ϕ are smaller than the symbol size. (B) The Binder cumulant for an $O(3)$ order parameter $C_B = 1 - \frac{3\langle \vec{\phi}^2 \rangle}{5\langle \vec{\phi}^4 \rangle}$, where $\vec{\phi} = \frac{1}{N} \sum_i \vec{\phi}_i$, approaching the expected values of 0.4 and 0 in the two phases. (C) Equal-time pairing cor-

relations in systems of size $L = 10, 12, 14$ and $\beta = L$ for each curve, as a function of r . Dashed (solid) lines show $\bar{P}_+$ ($\bar{P}_-$), corresponding to A_{1g} (B_{1g}) superconducting order parameters, in which the pairing amplitude in the two fermion flavors is of the same (opposite) sign, respectively. r_c is the estimated position of the SDW critical point.

The SDW critical modes mediate effective interfermion interactions, which can lead to instabilities of the Fermi surface. As a result, additional competing phases can appear. Near the SDW critical point, these instabilities are a result of a subtle competition between the enhancement of the SDW fluctuations, which tends to strengthen the effective interactions, and the loss of coherence of the fermionic quasiparticles (10, 11). Superconductivity is a natural candidate for the leading potential instability. To examine the emergence of a superconducting phase near the SDW critical point, we have computed equal-time pairing correlations $P_{\pm} = \langle \vec{x}_i | \Delta_{\pm}(\vec{x}_i) \Delta_{\pm}(0)^{\dagger} \rangle$. Here, $\Delta_{\pm}(\vec{x}_i) = i s_{ab}^j (\Psi_{ixa} \Psi_{ixb} \pm \Psi_{iyb} \Psi_{iyb})$ (where $a, b = \uparrow, \downarrow$ are spin indices) are superconducting order parameters with either a + or – relative sign between the two fermionic flavors (square lattice symmetry A_{1g} and B_{1g} , respectively).

To probe for long-range order, we measured $P_{\pm}(\vec{x}_i)$ near the maximum range $\vec{x}_{\max} = (L/2, L/2)$. We plot $\bar{P}_{\pm}(\vec{x}_{\max}) = \frac{1}{9} \sum_{\epsilon_{xy}=-1}^1 P_{\pm}(\vec{x}_{\max} + \epsilon_x \vec{\eta}_x + \epsilon_y \vec{\eta}_y)$, where $\vec{\eta}_x = (1, 0)$ and $\vec{\eta}_y = (0, 1)$, in Fig. 4C. Long-range superconducting order at $\beta \rightarrow \infty$ would correspond to superconducting correlations that saturate to a constant upon increasing L and β .

The B_{1g} pairing correlations are found to be significantly enhanced in the vicinity of the SDW critical point, $r_c \approx 0.25$. The A_{1g} correlations are significantly smaller in magnitude and negative in sign. This is consistent with the expectation that the effective attraction mediated by magnetic fluctuations promotes superconductivity with a sign change between the two orbitals (21, 22).

The maximum of the B_{1g} correlations occurs for $r \approx 0.5$, on the disordered side of the magnetic critical point, which is located at $r_c \approx 0.25$ (23). Notably, the suppression of the supercon-

ducting correlations away from the optimal r is very asymmetric: Whereas the pairing correlations decrease gradually for $r > r_c$, they are suppressed dramatically for $r < r_c$. This may be a result of the opening of an SDW gap on portions of the Fermi surface.

The method described here opens the way to study various physical aspects of spin density wave transitions in metals, in a numerically exact way. The interplay between unconventional superconductivity and magnetism and possible non-Fermi liquid behavior in the quantum critical regime should now be accessible. Moreover, such simulations will provide controlled benchmarks for analytic approximations (7, 8, 10, 11).

The two-band model presented here is a member of a wider family of strongly correlated fermionic models that can be rendered free of the sign problem. It has already been established that some models with two flavors of fermions interacting via a four-fermion interaction are sign problem free at generic fermion density (24). Notably, these models do not rely on any specific characteristic of the electron dispersion; e.g., there is no requirement for particle-hole symmetry, or any symmetry that relates the two bands. Extensions of this trick to related models of physical interest should be possible.

References and Notes

1. T. Helm *et al.*, *Phys. Rev. Lett.* **105**, 247002 (2010).
2. K. Hashimoto *et al.*, *Science* **336**, 1554 (2012).
3. T. Park *et al.*, *Nature* **440**, 65 (2006).
4. L. Balents, *Nature* **464**, 199 (2010).
5. M. Troyer, U.-J. Wiese, *Phys. Rev. Lett.* **94**, 170201 (2005).
6. J. A. Hertz, *Phys. Rev. B* **14**, 1165 (1976).
7. A. Abanov, A. V. Chubukov, *Phys. Rev. Lett.* **84**, 5608 (2000).
8. A. Abanov, A. Chubukov, *Phys. Rev. Lett.* **93**, 255702 (2004).

9. See supplementary material on Science Online.
10. M. A. Metlitski, S. Sachdev, *Phys. Rev. B* **82**, 075128 (2010).
11. S. A. Hartnoll, D. M. Hofman, M. A. Metlitski, S. Sachdev, *Phys. Rev. B* **84**, 125115 (2011).
12. C. Weber, K. Haule, G. Kotliar, *Phys. Rev. B* **82**, 125107 (2010).
13. L. Taillefer, *Annu. Rev. Condens. Matter Phys.* **1**, 51 (2010).
14. N. Doiron-Leyraud, L. Taillefer, *Physica C* **481**, 161 (2012).
15. G. Sordi, P. Sémon, K. Haule, A.-M. S. Tremblay, *Phys. Rev. Lett.* **108**, 216401 (2012).
16. E. Gull, O. Parcollet, A. J. Millis, Superconductivity and the pseudogap in the two-dimensional Hubbard model, arXiv:1207.2490.
17. A. Abanov, A. V. Chubukov, J. Schmalian, *Adv. Phys.* **52**, 119 (2003).
18. R. Blankenbecler, D. J. Scalapino, R. L. Sugar, *Phys. Rev. D Part. Fields* **24**, 2278 (1981).
19. S. R. White *et al.*, *Phys. Rev. B Condens. Matter* **40**, 506 (1989).
20. F. F. Assaad, H. G. Evertz, in *Computational Many-Particle Physics*, H. Fehske, R. Shneider, A. Weiße, Eds. (Springer, Berlin, 2008).
21. D. J. Scalapino, E. Loh Jr., J. E. Hirsch, *Phys. Rev. B Condens. Matter* **34**, 8190 (1986).
22. P. Monthoux, A. V. Balatsky, D. Pines, *Phys. Rev. Lett.* **67**, 3448 (1991).
23. E. G. Moon, S. Sachdev, *Phys. Rev. B* **82**, 104516 (2010).
24. Y. Motome, M. Imada, *J. Phys. Soc. Jpn.* **66**, 1872 (1997).

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Optomechanical Dark Mode

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Thermal mechanical motion hinders the use of a mechanical system in applications such as quantum information processing. Whereas the thermal motion can be overcome by cooling a mechanical oscillator to its motional ground state, an alternative approach is to exploit the use of a mechanically dark mode that can protect the system from mechanical dissipation. We have realized such a dark mode by coupling two optical modes in a silica resonator to one of its mechanical breathing modes in the regime of weak optomechanical coupling. The dark mode, which is a superposition of the two optical modes and is decoupled from the mechanical oscillator, can still mediate an effective optomechanical coupling between the two optical modes. We show that the formation of the dark mode enables the transfer of optical fields between the two optical modes. Optomechanical dark mode opens the possibility of using mechanically mediated coupling in quantum applications without cooling the mechanical oscillator to its motional ground state.

In an optomechanical resonator, circulating optical fields can couple to the motion of a mechanical oscillator via radiation pressure (1, 2). Studies of optomechanical interactions have led to the experimental realization of a number of remarkable phenomena, including strong coupling between an optical and a mechanical

mode (3–5), optomechanically induced transparency (OMIT) (5–7), and coherent interconversion between optical and mechanical excitations (4, 8). These advances have opened up avenues in coupling hybrid quantum systems, by taking advantage of unique properties of an optomechanical system, and especially in developing a

new type of light-matter quantum interface (9, 10). A major obstacle, however, is the inherent thermal motion of a mechanical oscillator. A straightforward, but technically challenging, approach to overcome the thermal motion is to cool the mechanical oscillator to its motional ground state (4, 11–13). An alternative approach, as proposed recently, is to exploit the use of a mechanically dark mode, which is decoupled from the mechanical oscillator and thus is robust against mechanical dissipation (14, 15). A similar process that exploits mechanical coupling, while circumventing effects of thermal mechanical motion, has also been explored for trapped ions (16, 17).

A dark mode is analogous to the well-known coherent-population-trapped state or the dark state in atomic physics (18, 19). Figure 1A illustrates a Λ -type three-level atomic system, in which the two lower states, $|1\rangle$ and $|2\rangle$, couple to an upper state via two dipole optical transitions. The

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formation of a dark state, which is a special coherent superposition of $|1\rangle$ and $|2\rangle$, prevents the optical excitation to the upper state through destructive interference, thus protecting the system from dissipation or decoherence associated with the upper state. In addition to atomic vapors, dark states have also been demonstrated in solid-state systems of single electron spins, such as quantum dots and diamond nitrogen vacancy (NV) centers (20–22).

Here, we report an experimental demonstration of a mechanically dark mode by coupling two optical whispering gallery modes (WGMs) to a mechanical breathing mode in a silica resonator in the regime of weak optomechanical coupling. This dark mode is a special coherent superposition of the two optical modes. The cancellation in the mechanical coupling induced by the superposition decouples the dark mode from the mechanical oscillator. The formation of the dark mode, however, also induces a conversion of optical fields from one optical mode to the other, effectively mediating an optomechanical coupling between the two optical modes. This type of mechanically mediated coupling can be immune to thermal mechanical motion, providing a promising mechanism for interfacing hybrid quantum systems (9, 14, 15).

To introduce the optomechanical dark mode, we consider an optomechanical system, in which two optical modes couple to a mechanical oscillator with optomechanical coupling rates G_1 and G_2 , respectively (Fig. 1B). As illustrated in Fig. 1C, the optomechanical coupling is driven by two strong laser fields, E_1 and E_2 , with frequencies, ω_{l1} and ω_{l2} , that are each one mechanical frequency, ω_m , below the respective cavity resonance, ω_1 and ω_2 . In analogy to atomic dark and bright states, we define a mechanically dark mode, $\hat{a}_D = (G_2\hat{a}_1 - G_1\hat{a}_2)/\tilde{G}$, and a mechanically bright mode, $\hat{a}_B = (G_1\hat{a}_1 + G_2\hat{a}_2)/\tilde{G}$, with $\tilde{G} = \sqrt{G_1^2 + G_2^2}$, where $\hat{a}_1$ and $\hat{a}_2$ are the annihilation operators for the signal field in the two optical modes in the respective rotating frame of the external driving field. The optomechanical interaction Hamiltonian in terms of these superposition optical modes is then given by

$$H = \hbar\omega_m(\hat{b}^\dagger\hat{b} + \hat{a}_B^\dagger\hat{a}_B + \hat{a}_D^\dagger\hat{a}_D) + \hbar\tilde{G}(\hat{a}_B^\dagger\hat{b} + \hat{a}_B\hat{b}^\dagger) \quad (1)$$

where $\hat{b}$ is the annihilation operator for the mechanical oscillator. As shown in Eq. 1, the dark mode is decoupled from the mechanical oscillator (14, 15). The dark mode is spectrally separated from the bright mode in the limit of ultrastrong optomechanical coupling, for which G_1 and G_2 far exceed κ_1 and κ_2 , the decay rates of mode 1 and mode 2, as well as γ_m , the mechanical damping rate. In this limit, the coupling between the bright mode and the mechanical oscillator leads to the formation of two normal modes with frequencies given by $\omega_m \pm \tilde{G}$.

In the limit of weak optomechanical coupling, the dark mode can no longer be spectrally sep-

arated from the bright mode. The system, however, can still be driven optically into the dark mode via suppression of the bright-mode excitation. In contrast to the dark mode, an optical excitation of the bright mode induces a mechanical excitation. Anti-Stokes scattering of the strong driving field off this mechanical excitation in turn generates an optical field that interferes destructively with the optical excitation field in the bright mode. This OMIT process can effectively prevent the excitation of the bright mode. Specifically, when the optomechanical system shown in Fig. 1B is excited by a signal field resonant with mode 1, the OMIT suppresses the bright-mode amplitude by a factor of $(1 + \tilde{C})$, where $\tilde{C} = C_1 + C_2$, with $C_i = 4G_i^2/\gamma_m\kappa_i$ ($i = 1, 2$) being the optomechanical cooperativity (23, 24). For simplicity, $\kappa_1 = \kappa_2$ is also assumed. The ratio of dark- to bright-mode population in the steady state is then given by $(G_2/G_1)^2(1 + \tilde{C})^2$ (24). Hence, a large cooperativity is sufficient in preventing the excitation of the bright mode via OMIT, effectively driving the system into the dark mode. Similar results can also be obtained when $\kappa_1 \neq \kappa_2$, with the dark-to-bright-population ratio modified as $(G_2/G_1)^2[1 + C_2 + C_1(\kappa_1/\kappa_2)]^2$ (24). In a typical optomechanical system, the optical linewidth is orders of magnitude greater than the mechanical linewidth. It is thus more practical to realize large cooperativity than ultrastrong coupling.

The dark mode can be probed through the excitation of the two individual optical modes. In the above case, the intracavity field amplitudes of mode 1 and mode 2 are, respectively,

$$a_1 = a_0[C_1/(1 + \tilde{C}) + C_2]/\tilde{C} \quad (2A)$$

$$a_2 = a_0\sqrt{C_1C_2}[1/(1 + \tilde{C}) - 1]/\tilde{C} \quad (2B)$$

where a_0 is the field amplitude in mode 1 in the absence of optomechanical coupling. In both equations, the first term in the bracket is due to the bright mode, and the second term is due to the dark mode (24). As expected from the suppression of the bright-mode amplitude by OMIT, the bright-mode term scales with $1/(1 + \tilde{C})$. Equation 2B also shows that the bright- and dark-mode contributions interfere destructively in mode 2. In this context, the excitation of mode 2 results directly from the suppression of the bright-mode amplitude.

We used silica microspheres with a diameter near 30 μm as a model optomechanical resonator (25). Two WGMs, with mode 1 near 637 nm and mode 2 near 800 nm, coupled to the (1, 0) mechanical breathing mode of a silica microsphere. Two samples were used, with $(\kappa_1, \kappa_2, \omega_m, \gamma_m)/2\pi \approx 19, 16, 150, 0.055$ MHz and $(\kappa_1, \kappa_2, \omega_m, \gamma_m)/2\pi \approx 15, 15, 154, 0.06$ MHz for sample A (used for Fig. 2) and B (used for Fig. 3), respectively. All experiments were carried out at room temperature.

For the demonstration of the dark mode, E_{in} with frequency ω_{in} excited mode 1 resonantly or near-resonantly. Optical emissions from mode 1 and mode 2, which are directly proportional to the respective intracavity intensity, were measured as a function of detuning, $\Delta = \omega_{in} - \omega_{l1}$, with a special heterodyne-detection technique (24). For simplicity, we refer to these spectra as emission spectra. To avoid heating induced by the strong driving fields and to enable measurements on the behavior of the mechanical mode, we used 8- μs -long optical pulses for E_1 , E_2 , and E_{in} , each with the same timing and with a duty cycle below 5%. Figure 1D shows a simplified schematic of the experimental setup. In order to probe the steady-state behavior, emission spectra

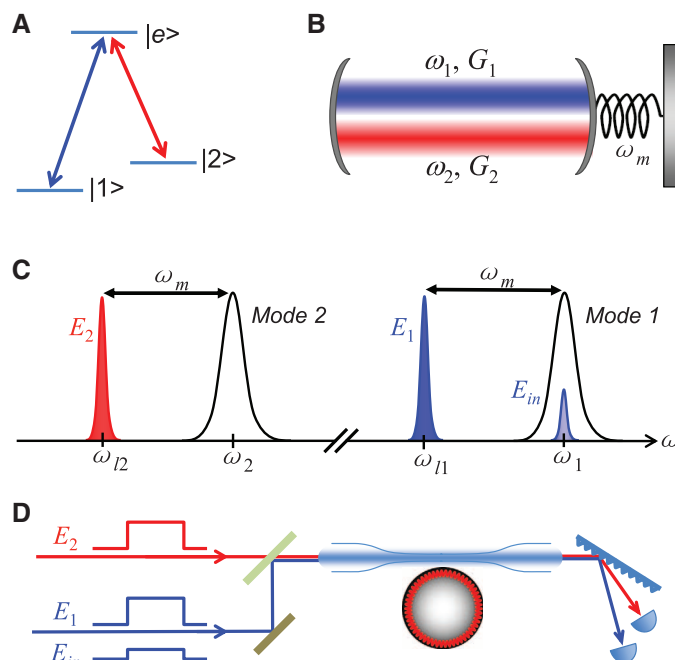


Fig. 1. Concept of the experiment. (A) A Λ -type three-level system that can lead to the formation of a dark state. (B) An optomechanical system in which two optical modes couple to a mechanical oscillator via radiation pressure, with respective optomechanical coupling rates G_1 and G_2 . (C) Two optical fields, E_1 and E_2 , at the red side band of the respective optical resonance drive the respective optomechanical coupling. (D) A simplified schematic of the experimental setup, with E_{in} exciting mode 1 in a silica microsphere.

were obtained with time-gated detection, with a 1- μ s detection gate positioned between 6 and 7 μ s of the incident optical pulses (Fig. 2E inset) (24). At relatively high optical powers, spectral shifts of WGM resonances resulting from Kerr effects become substantial. For experiments in Fig. 2, care was taken to keep the frequencies of the two driving fields at ω_m below the respective WGM resonances.

Figure 2A shows emission spectra from mode 1, obtained with $C_1 = 1.4$ and $C_2 = 0$. In this case, the mechanical oscillator couples only to mode 1. The resulting OMIT process prevents the excitation of mode 1, inducing a sharp dip at the anti-Stokes resonance, $\Delta = \omega_m$, with a width determined by $\gamma_m(1 + C_1)$ (6, 7). For our studies,

C_1 was determined from theoretical fitting of OMIT dips obtained with $C_2 = 0$, whereas C_2 was similarly determined from theoretical fitting of OMIT dips obtained with $C_1 = 0$ and with mode 2 excited resonantly by an input signal field.

By tuning on both E_1 and E_2 , we coupled both optical modes to the mechanical oscillator. With increasing C_2 , the excitation of the dark mode should lead to an increasing excitation of mode 1 and thus the vanishing of the OMIT dip for mode 1 (see also Eq. 2A). Figure 2B shows emission spectra from mode 1 obtained with $C_1 = 1.4$ and with increasing C_2 . The depth of the dip at $\Delta = \omega_m$ decreases with increasing C_2 , accompanied by a spectral broadening of the dip. Figure

2B also shows a slight spectral shift of the emission dip at relatively high C_2 . The shift is due to the optical spring effect, for which radiation pressure induces a shift in ω_m .

The dark-mode formation necessitates the conversion of optical fields from mode 1 to mode 2, because E_m couples directly only to mode 1. Figure 2C shows the emission spectra from mode 2 obtained under nearly the same condition as that for Fig. 2B. At $\Delta = \omega_m$, the emission from mode 2 increases simultaneously with the emission from mode 1 with increasing, but still relatively small C_2 (Fig. 2D), which is a signature that the system is driven toward a dark mode.

For energy conservation, the optical mode conversion should induce a dip in the emission spectrum of mode 1. A pronounced dip in the emission spectra of mode 1 persists even at the highest C_2 used (Fig. 2B). Under these conditions the system is nearly completely in the dark mode. With increasing C_2 , the dip in the emission spectra of mode 1 evolves from an OMIT dip (at $C_2 = 0$) into a dip that reflects the process of optical mode conversion.

For a quantitative analysis, we used the coupled oscillator model to describe the coupling between the mechanical oscillator and the two optical modes (24). The solid curves in Fig. 2, A to C, show the calculated emission spectra from mode 1 and mode 2, with all parameters determined directly ($\kappa_1, \kappa_2, \omega_m, \gamma_m$) or indirectly ($C_1, C_2, \eta_1\eta_2 = 0.16$) from experiments, with η_1 and η_2 being the output coupling ratio for the two optical modes. Figure 2D plots the calculated emission power at $\Delta = \omega_m$ for the two optical modes. Additional theoretical calculations also confirm that the experimental results shown in Fig. 2 reflect the steady-state behavior of the optomechanical system (24).

The agreement between experiment and theory shown in Fig. 2, A to D, enables us to determine the dark-mode fraction (the ratio of the dark-mode population over the total bright- and dark-mode population) by using the coupled oscillator model. The steady-state dark-mode fraction corresponding to the experimental results in Fig. 2D is calculated and plotted (Fig. 2E). With $C_1 = 1.4$ and $C_2 = 3.5$, the dark-mode fraction reaches 99%.

The excitation of the dark mode not only leads to the simultaneous rise of optical emissions from mode 1 and mode 2 with increasing (but relatively small) C_2 , as discussed earlier, but also accounts for the saturation of the optical mode conversion observed at relatively large C_2 . As shown in Fig. 2D, after the system is driven into a predominantly dark mode, a further increase in C_2 leads to a saturation and then decrease in the emission from mode 2, whereas the emission from mode 1 continues to rise.

Dark-mode formation can enable efficient transfer of optical fields between the two optical modes. The overall photon-conversion efficiency, defined as the ratio of the output-signal photon flux for

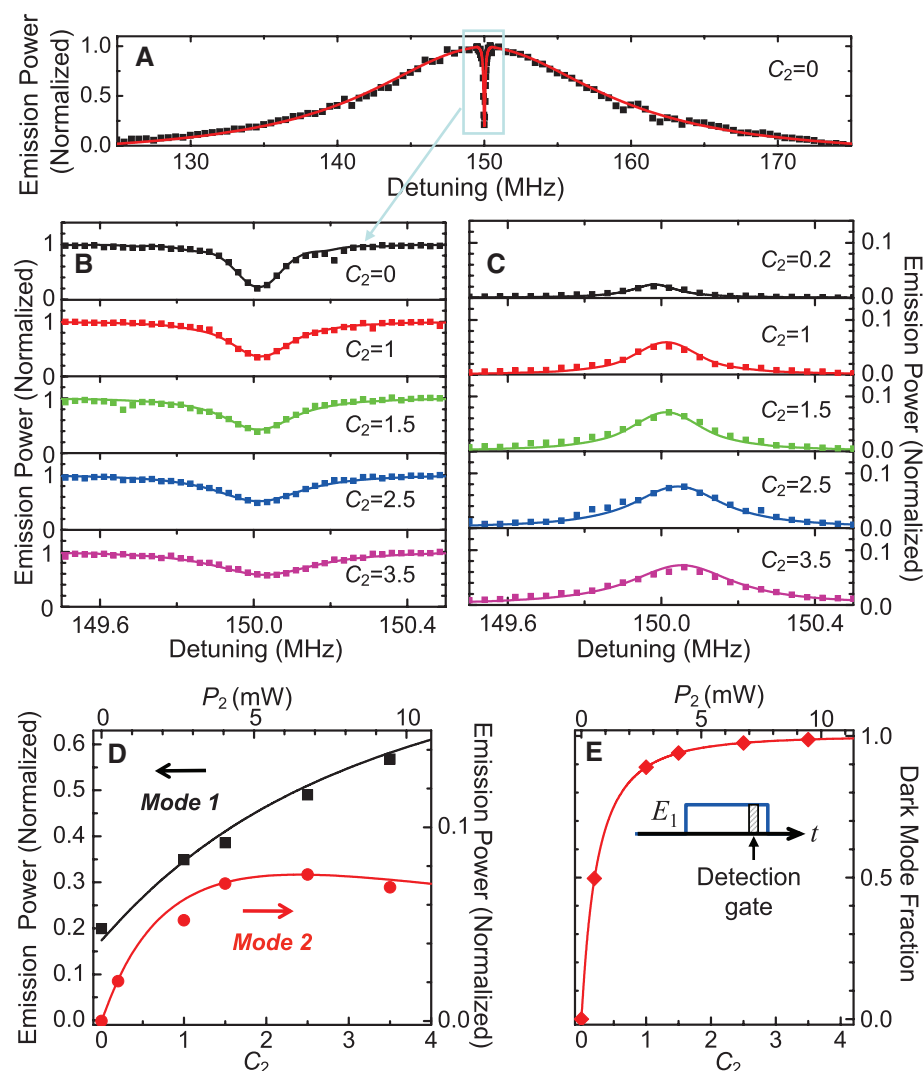


Fig. 2. Excitation of the dark mode. (A and B) Optical emission from mode 1 as a function of detuning, $\Delta = \omega_m - \omega_{L1}$, with $C_1 = 1.4$ ($P_1 = 2.5$ mW) and $P_{in} = 10$ μ W. The emission power is normalized to that obtained at the cavity resonance with $C_1 = C_2 = 0$. (C) Optical emission from mode 2 as a function of Δ with $C_1 = 1.4$ and $P_{in} = 20$ μ W. Care was taken in normalizing the emission power to the input signal power (24). (D) Emission powers from mode 1 (squares) and mode 2 (circles) at $\Delta = \omega_m$ as a function of C_2 , derived from (B) and (C). Solid lines in (A) to (D) are the theoretical calculations discussed in the text. (E) Calculated dark-mode fraction. The diamonds correspond to the experimental results shown in (D). (Inset) The timing of the detection gate used for the experiment. P_{in} , P_1 , and P_2 are incident optical powers for E_{in} , E_1 , and E_2 , respectively.

Fig. 3. Heterodyne-detected optical emission from mode 2 obtained with $P_{\text{in}} = 0.1$ mW, $C_1 = 0.25$, and $C_2 = 0.4$. A driving field at the red sideband of mode 2 served as the local oscillator. The dashed line plots the calculated envelope for the heterodyne signal, with an adjustable offset. (**Inset**) The beat signal (squares) with an expanded time scale. Solid red line shows for reference a periodic oscillation with $\omega_m/2\pi = 154$ MHz.

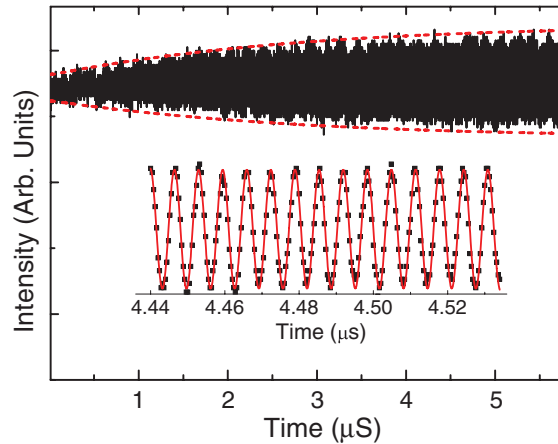
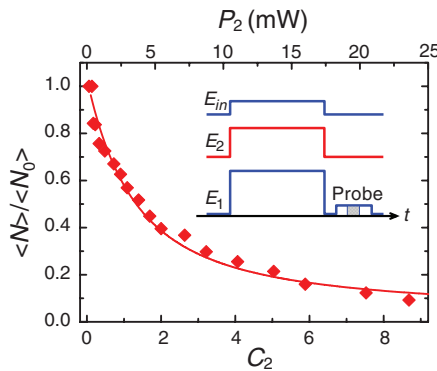


Fig. 4. Induced mechanical excitation underlying the OMIT for the bright mode, obtained as a function of C_2 and with $C_1 = 0.7$ ($P_1 = 1.25$ mW) and $P_{\text{in}} = 10$ μ W. At $C_2 \ll 1$, $(\omega_1 - \omega_L)/2\pi$ and $(\omega_2 - \omega_{L2})/2\pi$ are estimated to be 150 and 145 MHz, respectively. The solid line shows the result of the theoretical calculation, as discussed in the text. (**Inset**) The pulse sequence used, with the shaded area indicating the timing of the detection gate.



mode 2 over the input-signal photon flux for mode 1, is given by $\chi = 4\eta_1\eta_2C_1C_2/(1 + C_1 + C_2)^2$ (14, 15, 24). Near-unity photon conversion can thus be achieved in the limit that $C_1 = C_2 \gg 1$ and $\eta_1 = \eta_2 = 1$. With $C_1 = C_2 \gg 1$, the dark mode features nearly equal photon populations in the two optical modes. Unity photon conversion can occur because a destructive interference prevents the escape of photons from mode 1 (14). The small output-coupling ratio ($\eta_1\eta_2 = 0.16$), along with the modest cooperativity used in our experiment, leads to the relatively small mode-conversion efficiency observed in Fig. 2.

The optical-mode conversion can also be described theoretically and completely with a scattering matrix approach and without resorting to the dark-mode concept (26, 27). In this approach, the condition of $C_1 = C_2 \gg 1$ can be understood simply in terms of impedance matching (26). By establishing a close connection between the weak and strong coupling regime, the dark-mode description provides important insights on why the mode-conversion process can be robust against thermal mechanical noise even in a weak coupling regime.

We further characterized the emission from mode 2 by measuring directly in the time domain the heterodyne signal that mixes the emission from mode 2 with a driving field E_2 . Figure 3 shows the transient heterodyne signal obtained with $C_1 = 0.25$ and $C_2 = 0.4$. The rise of the heterodyne signal with a rise time of order $1/[(1 + C_1 + C_2)\gamma_m]$ is in good agreement with

the theoretical calculation based on the coupled oscillator model and on the use of the experimentally determined C_1 , C_2 , and γ_m . The heterodyne signal features a periodic oscillation with a frequency given by ω_m (Fig. 3 inset), demonstrating the coherent nature of the optical mode conversion. Specifically, there is a well-defined relative phase between E_2 and the converted optical field in mode 2.

We now turn to the behavior of the mechanical oscillator, which can serve as a probe for the OMIT process for the bright mode when the optical excitation is dominated by the dark mode. As discussed earlier, the OMIT arises from anti-Stokes scattering of the driving fields off the mechanical excitation induced by the bright mode excitation. To probe the mechanical excitation, we added a weak 3- μ s probe pulse, which arrives 1 μ s after E_1 and is also at the same frequency as E_1 (Fig. 4 inset). We used the probe pulse and time-gated detection, with the 1- μ s gate positioned at the center of the probe pulse, to measure the displacement power density spectrum of the mechanical mode (24). The spectrally integrated area of the power density spectrum determines the average phonon number, $\langle N \rangle$, of the mechanical mode. For the experiment, a relatively strong input signal was used such that $\langle N_0 \rangle$, the average phonon number obtained with $C_2 = 0$, is two orders of magnitude greater than the average thermal phonon number.

$\langle N \rangle / \langle N_0 \rangle$ obtained with $C_1 = 0.7$ were plotted (Fig. 4) as a function of C_2 , for which sample

A was used, and ω_{11} and ω_{12} were fixed and were near the respective red sideband. Other experimental conditions are the same as those for Fig. 2D. The experimental results are in good agreement with the theoretical calculation based on the coupled oscillator model. The calculation also includes corrections due to the Kerr effect with $\epsilon_i = \xi_i P_2$ ($i = 1, 2$), where ϵ_1 and ϵ_2 are the Kerr shift for mode 1 and mode 2 induced by E_2 , respectively, and $(\xi_1, \xi_2) = (-0.1, -0.46)$ MHz/mW. The observation of the induced mechanical excitation when the system is predominantly in the dark mode confirms the underlying OMIT process for the bright mode. Figure 4 also shows that the anti-Stokes scattering of E_2 damps the mechanical oscillation when the system is driven to the dark mode with increasing C_2 .

Although silica WGM resonators feature modest optomechanical cooperativity, much greater cooperativity (10^3 or greater) can be attained with membrane- or nanobeam-based optomechanical systems that feature ultrahigh mechanical Q factors (28, 29). With these systems, mechanically mediated processes, such as the optical mode conversion, can be pursued in a quantum regime at an elevated temperature. The concept of the dark mode can also be extended to other hybrid mechanical systems (30, 31), including the recently developed system of a mechanical resonator coupling to a single-electron spin in a diamond NV center (32).

Note added in proof: After the acceptance for publication of this work, optical mode conversion in an optomechanical crystal cavity was reported by Hill *et al.* (33).

References and Notes

1. T. J. Kippenberg, K. J. Vahala, *Science* **321**, 1172 (2008).
2. M. Aspelmeyer, P. Meystre, K. Schwab, *Phys. Today* **65**, 29 (2012).
3. S. Gröblacher, K. Hammerer, M. R. Vanner, M. Aspelmeyer, *Nature* **460**, 724 (2009).
4. E. Verhagen, S. Deléglise, S. Weis, A. Schliesser, T. J. Kippenberg, *Nature* **482**, 63 (2012).
5. J. D. Teufel *et al.*, *Nature* **471**, 204 (2011).
6. S. Weis *et al.*, *Science* **330**, 1520 (2010); 10.1126/science.1195596.
7. A. H. Safavi-Naeini *et al.*, *Nature* **472**, 69 (2011).
8. V. Fiore *et al.*, *Phys. Rev. Lett.* **107**, 133601 (2011).
9. K. Stannigel, P. Rabl, A. S. Sørensen, P. Zoller, M. D. Lukin, *Phys. Rev. Lett.* **105**, 220501 (2010).
10. L. Tian, H. L. Wang, *Phys. Rev. A* **82**, 053806 (2010).
11. A. D. O'Connell *et al.*, *Nature* **464**, 697 (2010).
12. J. D. Teufel *et al.*, *Nature* **475**, 359 (2011).
13. J. Chan *et al.*, *Nature* **478**, 89 (2011).
14. Y. D. Wang, A. Clerk, *Phys. Rev. Lett.* **108**, 153603 (2012).
15. L. Tian, *Phys. Rev. Lett.* **108**, 153604 (2012).
16. A. Sørensen, K. Molmer, *Phys. Rev. Lett.* **82**, 1971 (1999).
17. C. A. Sackett *et al.*, *Nature* **404**, 256 (2000).
18. E. Arimondo, *Prog. Opt.* **35**, 257 (1996).
19. M. D. Lukin, *Rev. Mod. Phys.* **75**, 457 (2003).
20. X. D. Xu *et al.*, *Nat. Phys.* **4**, 692 (2008).
21. C. Santori *et al.*, *Phys. Rev. Lett.* **97**, 247401 (2006).
22. E. Togan, Y. Chu, A. Imamoglu, M. D. Lukin, *Nature* **478**, 497 (2011).
23. Y. D. Wang, A. Clerk, *N. J. Phys.* **14**, 105010 (2012).
24. Materials and methods are available as supplementary materials on Science Online.

25. Y. S. Park, H. L. Wang, *Nat. Phys.* **5**, 489 (2009).
 26. A. H. Safavi-Naeini, O. Painter, *N. J. Phys.* **13**, 013017 (2011).
 27. D. E. Chang, A. H. Safavi-Naeini, M. Hafezi, O. Painter, *N. J. Phys.* **13**, 023003 (2011).
 28. J. D. Thompson *et al.*, *Nature* **452**, 72 (2008).
 29. G. Anetsberger *et al.*, *Nat. Phys.* **5**, 909 (2009).
 30. P. Rabl *et al.*, *Nat. Phys.* **6**, 602 (2010).
 31. P. Treutlein, D. Hunger, S. Camerer, T. W. Hänsch, J. Reichel, *Phys. Rev. Lett.* **99**, 140403 (2007).
 32. S. Kolkowitz *et al.*, *Science* **335**, 1603 (2012); 10.1126/science.1216821.
 33. J. T. Hill, A. H. Safavi-Naeini, J. Chan, O. Painter, *Nat. Comm.* **3**, 1196 (2012).

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Porphyry-Copper Ore Shells Form at Stable Pressure-Temperature Fronts Within Dynamic Fluid Plumes

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Porphyry-type ore deposits are major resources of copper and gold, precipitated from fluids expelled by crustal magma chambers. The metals are typically concentrated in confined ore shells within vertically extensive vein networks, formed through hydraulic fracturing of rock by ascending fluids. Numerical modeling shows that dynamic permeability responses to magmatic fluid expulsion can stabilize a front of metal precipitation at the boundary between lithostatically pressured up-flow of hot magmatic fluids and hydrostatically pressured convection of cooler meteoric fluids. The balance between focused heat advection and lateral cooling controls the most important economic characteristics, including size, shape, and ore grade. This self-sustaining process may extend to epithermal gold deposits, venting at active volcanoes, and regions with the potential for geothermal energy production.

Porphyry-type ore deposits are among the world's premier metal resources, supplying most of the copper, molybdenum, and a substantial part of gold production (1). They form in response to focused expulsion of metal-bearing saline fluids from large chambers of cooling hydrous magma (2). A restricted zone of ore-mineral precipitation within a more extensive plume of fluid up-flow is thought to be the key to economic metal accumulation (3). The ore typically forms well-defined bodies of bell-like or cylindrical shape, centered on dike- or stocklike porphyry intrusions at some distance above cupolas in the roof of magma chambers (Fig. 1) (1, 4–7). Geological observations and fluid inclusion data indicate physical and chemical processes of a dynamic flow system that resemble volcanic systems at the verge of eruption (8, 9).

The top of the ore shell is commonly abrupt and coincides with the top of a dense vein network (1), indicating that fluid pressures were sufficient to induce hydraulic fracturing. The core within and below the ore shell is veined but barren and commonly hosts fluid inclusions with intermediate density, interpreted to represent a hot (>600°C), near-lithostatically pressured single-phase fluid that ascended from the subjacent magma chamber (7). On ascent, this primary mag-

matic fluid separates into a low-salinity vapor and a high-salinity liquid phase, recorded by ubiquitous vapor and brine inclusions in all porphyry deposits (1, 7). The physical mechanism creating a zone of localized chemical precipitation during the lifetime of the system is key to metal enrichment to economic grades but remains elusive.

Here, we present numerical simulations of the physical hydrology of porphyry systems that link the expulsion of saline magmatic fluids with the transient evolution of rock permeability and the dynamics of two-phase fluid flow (10). The ge-

ometric configuration of our model is a two-dimensional (2D) representation of a 10- by 3-km magma chamber of elliptical shape with a cupola in the roof at 5 km depth (Fig. 2A). It has been constructed to resemble the dimensions of the Yerington porphyry system (Fig. 1B), which shows the most complete and best mapped exposure from a deep source pluton to the paleosurface. We compute the expulsion rates to be proportional to the rate of magma crystallization and release saline magmatic fluids through the cupola zone of a 3D magma chamber (10) (fig. S1).

Our model is based on published constitutive relationships and empirical parameterizations of the effects of brittle, ductile, and elastic rock mechanics on crustal permeability in a continuum porous-media approach (Fig. 2) (10). The model links a critically stressed crust with an average depth-dependent permeability profile (11, 12). Superimposed on this background behavior, permeability closes at temperatures above the brittle-ductile transition (13, 14) and opens at fluid pressures exceeding a criterion for rock failure (15), which varies from near-hydrostatic for brittle rock to near-lithostatic for ductile rock (Fig. 2). The model generates a dynamic hydrothermal system with varying domains of brittle and ductile rock behavior, solely from these initial conditions and geologically realistic descriptions of material properties.

The simulations demonstrate a self-sustaining mechanism that can stabilize a front of copper precipitation in porphyry deposits, which is con-

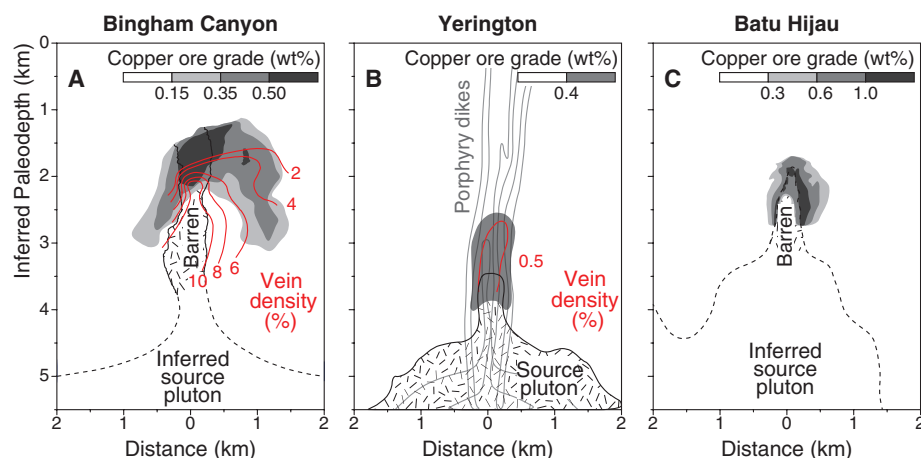


Fig. 1. Characteristic ore shells and vein densities of porphyry-style deposits. (A) Bingham Canyon, Utah, USA (6, 7). (B) Yerington, Nevada, USA (5). (C) Batu Hijau, Indonesia (4). Figures are simplified from published field observations and reconstructed geology.

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sistent with their characteristic zoning patterns. The interplay of permeability, heat advection, and nonlinear fluid properties establishes a vertically extensive up-flow zone of magmatic fluids (Fig. 3). It is characterized by a hot inner part with near-lithostatic pressures, which is overlain and surrounded by cooler parts with meteoric water convecting under hydrostatic conditions. A self-sealing rim of low permeability develops in response to the opposing permeability effects of the two fluid regimes. In the inner part, high temperatures above the brittle-ductile transition are maintained by the continuous supply of hot magmatic fluid, lowering permeability and keeping fluid pressures high. From outside, convection of meteoric fluids removes heat and shapes the sides of the plume. Their subvertical orientation is the result of an interplay of fluid and rock at temperatures between 350° and 400°C, at which the brittle-ductile transition starts (16) and strong gradients in fluid properties tend to maximize vertical, advective heat transport (17, 18). Heat transfer from the inner part of the plume to the convecting meteoric fluids on the sides is mainly by conduction through the low-permeability transition zone. Toward the upper part of the plume, meteoric convection becomes more intense in response to the increase in permeability of the host rock along the depth-dependent background profile (Fig. 2D) and leads to formation of the rounded top of the high-pressure, high-temperature part of the plume (Fig. 3A).

During volatile expulsion, magmatic fluids move through the hot plume in rapid overpressure-permeability waves (Fig. 3). At the injection location above the cupola, permeability is insufficient to accommodate the supply of magmatic volatiles at pressures below the failure criterion, which leads to wavelike behavior when applying an incremental hydrofracturing model that relates permeability changes to the amount of overpressure (10, 19). Even though our model does not explicitly include permeability anisotropy, a vertical movement of the overpressure-permeability waves develops naturally because the strongest (lithostatic) fluid pressure gradient is oriented vertically (movie S1). Inside the plume, the fluid-phase state fluctuates between single-phase fluid of intermediate density and high-pressure phase separation into vapor and liquid (Fig. 4A). Oscillatory patterns of quartz dissolution and precipitation have been interpreted to reflect pressure oscillations in several deposits (20). In parts of the plume, fluids become halite saturated, explaining correlations of salinities with sodium/potassium ratios of brine inclusions, which reveal that ore fluids were at least temporarily halite saturated in some porphyry deposits (21).

The location, shape, and thermal and pressure structure of the plume remained essentially stable over 50,000 years while fluids drained from the crystallizing magma chamber (fig. S3). Conditions in the upper parts closely resemble those inferred for porphyry copper ore deposition: When a magmatic fluid pulse crosses the transition zone

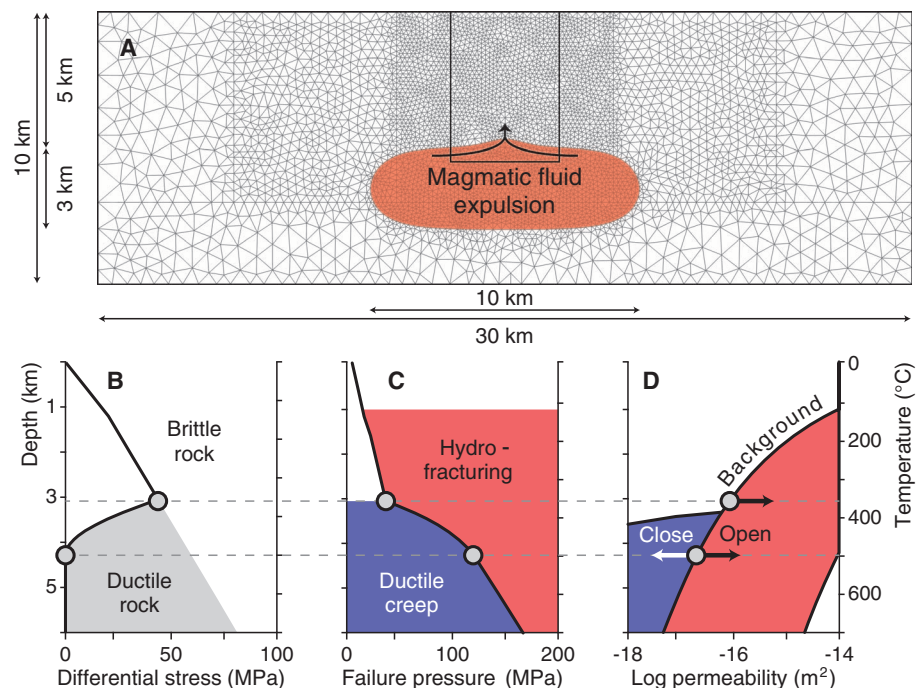


Fig. 2. Model configuration. (A) The mesh consists of ~9000 triangular elements with the finest resolution (50 m) above the magma chamber. The box in the center marks the location of the 4- by 5.5-km excerpt in Figs. 3A and 4. (B) The crust is assumed to be near-critically stressed (12); differential stress increases with depth (left axis). The stress field is not varied in our model but responds to thermal conditions so as to mimic the transition from brittle to ductile rock (13), exemplified with a schematic temperature gradient reaching magmatic temperatures (right axis). (C) Failure pressure defines a stress-state-dependent criterion for rock failure (15). Fluid pressures exceeding failure pressure will hydrofracture the rock (red). Ductile creep dominates at elevated temperatures and fluid pressures below this failure criterion (blue). (D) Background permeability follows a depth-dependent profile for average continental crust (11). Permeability opens incrementally during hydrofracturing (black arrow) and is reduced to the background value after overpressure release. At temperatures above the brittle-ductile transition, permeability closes, mimicking the loss of interconnected pore space due to increased ductile behavior (white arrow) (14). Elevated fluid pressures counteract this thermal effect, ensuring that hydrofracturing always starts from the background value whenever fluid pressure reaches the stress-state-dependent failure criterion (gray dots) (fig. S2) (10).

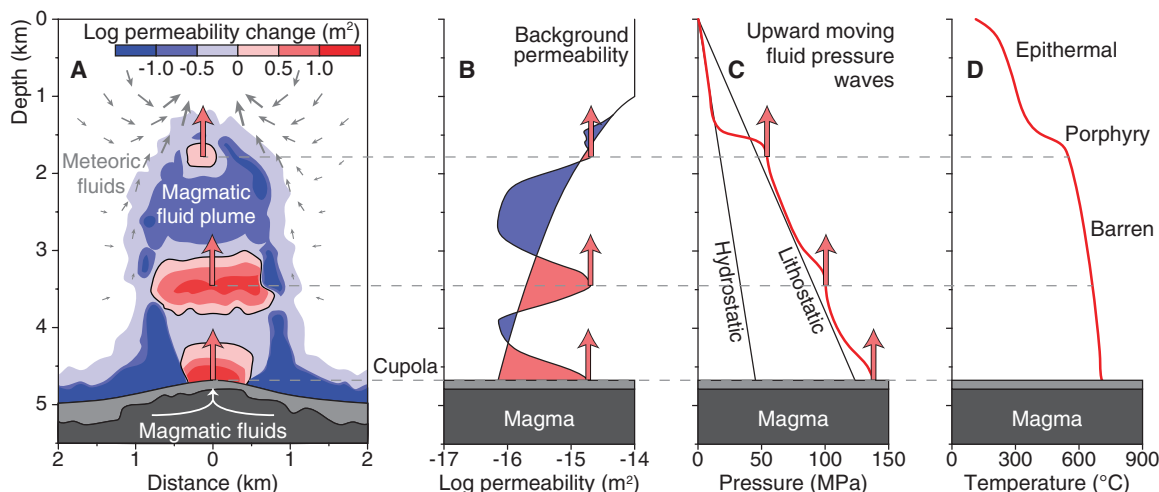
between the magmatic and meteoric fluid regimes, pressure drops from above lithostatic to hydrostatic, and temperature decreases from above 500°C to below 350°C within about 200 m (Fig. 3, C and D).

To explore the first-order chemical consequences of this physical process, we defined proxies for copper ore precipitation and quartz vein densities. Experimental data and fluid inclusion studies show that solubilities of copper drop sharply over the pressure and temperature gradients that emerge at the simulated transition zone (9, 22). A copper-enrichment potential for each rock volume was therefore computed by integrating all steps of fluid throughput in which a packet of magmatic fluid cooled through the temperature interval from 450° to 350°C, assuming 100% precipitation, and normalizing this integral to the copper content of the fluid released by a unit volume of source magma (Fig. 4B). Enrichment by a factor of 1000 can be achieved, corresponding to a maximum ore grade of about 2.5 weight percent if a concentration of 500 parts per million extractable copper in the source fluid

is assumed (10). This proxy provides an upper limit for the natural process, considering that copper extraction at the source may not be complete, and deposition in the ore shell may be somewhat dispersed in a heterogeneously fractured rock mass. The predicted ore shells, coinciding with the areas of sharp gradients in pressure and temperature within the central zone of greatest up-flow, closely resemble the bell-shape of many porphyry ore shells, including their characteristic sharp variations of ore grade near the top and the more gradual decrease down their limbs (Fig. 1A).

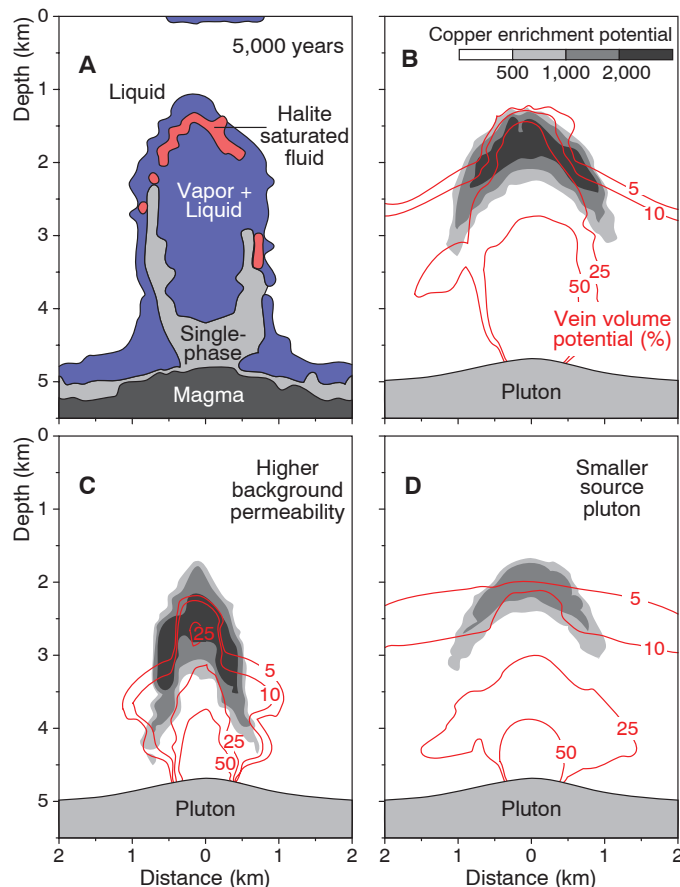
A proxy for quartz vein-volume can be calculated by integrating all steps of permeability increase, converting it into a pore space increase by using a cubic law relation (10). We did not model chemical precipitation but assumed that this space would be filled by quartz, which makes this proxy an upper limit for actual vein density. The modeled patterns of the vein-volume potential exhibit the characteristic relation to the ore shells as seen in natural systems (Figs. 1 and 4B). High vein density overlaps with predicted copper enrichment near the sharp top and upper flanks of

Fig. 3. Model results after 5000 years when a magmatic fluid plume with a stable temperature-pressure front is established. **(A)** Magmatic fluids ascend with permeability-overpressure waves (red), while heat removal by steady convection of meteoric fluids (gray arrows) focuses and stabilizes the plume. Fluid pulses travel through the lithostatically pressured part within a few years; the exact period depends on the parameterization of hydrofracturing and fluid supply rates (10). **(B)** Permeability waves reflect the responses of the rock to temperature and fluid pressure (Fig. 2). **(C)** Fluid pressure distribution varies from essentially lithostatic in the inner part to hydrostatic in the uppermost part. **(D)** Temperatures and pressures in the dynamic interior of



the magmatic plume are too high for ore mineralization (barren core), until a sharp drop along a near-stationary front creates the environment for porphyry-type ore formation, followed by further cooling to epithermal conditions.

Fig. 4. Fluid phase states and integrated mineralization predicted by the model. **(A)** Hot and lithostatically pressured primary fluids are injected into the host rock as a single-phase ("supercritical") fluid of intermediate density, then phase-separate into highly saline liquid and vapor on ascent and locally saturate solid halite because of the drop in temperature and pressure (Fig. 3). Precipitated halite is modeled as immobile and hence will accumulate and partially block pore space until it is dissolved again, either by surrounding fluids or by temperature or pressure changes (10). **(B)** Modeled copper-enrichment potential and vein-volume potential, integrated over the simulation period, serve as proxies for ore shells and vein densities. Models **(C)** and **(D)** illustrate variations in permeability and fluid source rate.



the ore shells but extends and increases to greater depth into the barren core, which is consistent with observations at Bingham Canyon (6), Bajo de la Alumbrera (23), and many other deposits.

The temporal relations between the quartz and copper proxies also resolve previously un-

explained field observations. The bulk of the vein-volume potential is established rapidly during the initial simulation stages, whereas the copper-enrichment potential accumulates more gradually after the magmatic fluid plume has stabilized (movie S2). This prediction describes a physical process

for observations showing that copper deposition is texturally later than the main mass of vein quartz (7) and with indications that early quartz veining with related potassic alteration acted on shorter time scales (24). As the fluid supply slowly decreases with the crystallization of the pluton, the magmatic fluid plume gradually retreats (fig. S3), controlling the vertical extent of the ore shell (Fig. 4B).

To test the sensitivity of the results to geologic factors, we modified the simulation configuration, first by varying permeability (Fig. 4C). Increasing background permeability by one order of magnitude results in a narrower and vertically more extensive enrichment zone. The downward limbs start to merge and may eventually result in a near-cylindrical ore body comparable with the one at Yerington (Fig. 1B). In a second variation, with a source pluton of half the size but identical geometry in our 2D profile (10), the resultant copper-enrichment and vein-volume potentials are reduced but show the same patterns and aspect ratios (Fig. 4, B and D).

These variations suggest that the width of the barren core develops independently from the width of the injection zone of the cupola (600 m) and that the host rock permeability has a stronger effect on narrowing this zone than does a reduced fluid supply rate. The width and height of the up-flow result from self-organization of the system. The vertical extent of the high-permeability profile is especially remarkable. Higher background permeabilities result in a more effective cooling by more vigorous external convection, and magmatic fluids can be expelled faster, eventually resulting in a stronger focusing effect. This explains why ore bodies are typically centered on porphyry stocks, without showing a correlation between deposit size and the dimension of these small intrusions (1).

The time scale of ore formation is between 50,000 and 100,000 years, which is in good agreement with geochronology (25) and earlier modeling

studies (14). The resulting fluid production rate of a few tens of kilograms per second is at the lower end of active volcanic systems that have been proposed to resemble ore-forming systems (26). The mechanism of fluid extraction from the magma, however, remains a major unknown in the model. Fluids may accumulate within the chamber beneath an impermeable carapace at the cupola and get episodically ejected together with magma by repeated porphyry-dike injections, which have been observed in many deposits (1, 5, 23).

In the hydrostatically pressured region above the porphyry ore shell, pulses of magmatic vapor episodically condense into the surrounding fluids and mix in variable proportions. This 100° to 400°C area is at conditions near the boiling curve of saline liquid and switches between a single-phase liquid state (as in Fig. 4A) and a continuous two-phase zone with locally restricted, vapor-dominated fluid lenses rising to the surface. Predicted temperature and pressure conditions are characteristic for acid alteration that overlaps with the tops of some porphyry deposits (1) and provide a physical link to epithermal gold mineralization (Fig. 3D) (27). These vein deposits often show evidence for episodic boiling events, and gold precipitation is confined to thin layers (28), possibly correlating with minor admixing of magmatic fluid pulses in the simulations.

The self-stabilizing process of focused fluid release from large magma chambers also sheds new light on the hydrological dynamics of active volcanoes and sources of geothermal energy. Ex-

cess degassing, whereby the amount of released volatiles exceeds the original volatile content of the erupted magma and volcanic conduit, requires fluid focusing from a large degassing magma chamber into a small area of venting (29). Quantifying the influence of the brittle-ductile transition on fluid flow is important for the characterization of high-enthalpy geothermal systems (14). On the other hand, cooler enhanced geothermal systems are produced through creating permeability by stimulating fluid overpressure, which is similar to vein formation in our porphyry model (30). More generally, our study supports the interpretation of permeability as a dynamic parameter that is determined by an intimate interplay of fluid properties, heat advection, and rock mechanics.

References and Notes

1. R. H. Sillitoe, *Econ. Geol.* **105**, 3 (2010).
2. J. W. Hedenquist, J. B. Lowenstern, *Nature* **370**, 519 (1994).
3. R. W. Henley, A. McNabb, *Econ. Geol.* **73**, 1 (1978).
4. J. Arif, T. Baker, *Miner. Depos.* **39**, 523 (2004).
5. J. H. Dilles, *Econ. Geol.* **82**, 1750 (1987).
6. G. Gruen, C. A. Heinrich, K. Schroeder, *Econ. Geol.* **105**, 69 (2010).
7. P. B. Redmond, M. T. Einaudi, E. E. Inan, M. R. Landtwing, C. A. Heinrich, *Geology* **32**, 217 (2004).
8. R. J. Bodnar et al., *Geology* **35**, 791 (2007).
9. M. R. Landtwing et al., *Earth Planet. Sci. Lett.* **235**, 229 (2005).
10. Materials and methods are available as supplementary materials on Science Online.
11. S. E. Ingebritsen, C. E. Manning, *Geofluids* **10**, 193 (2010).
12. M. D. Zoback, J. Townend, B. Grollmund, *Int. Geol. Rev.* **44**, 383 (2002).
13. R. O. Fournier, *Econ. Geol.* **94**, 1193 (1999).
14. D. O. Hayba, S. E. Ingebritsen, *J. Geophys. Res.* **102**, (B6), 12235 (1997).

15. S. F. Cox, *Geofluids* **10**, 217 (2010).
16. L. M. Cathles, *Econ. Geol.* **88**, 1977 (1993).
17. D. Coumou, T. Driesner, C. A. Heinrich, *Science* **321**, 1825 (2008).
18. T. Jupp, A. Schultz, *Nature* **403**, 880 (2000).
19. S. A. Rojstaczer, S. E. Ingebritsen, D. O. Hayba, *Geofluids* **8**, 128 (2008).
20. B. G. Rusk, M. H. Reed, *Geology* **30**, 727 (2002).
21. P. L. Cloke, S. E. Kesler, *Econ. Geol.* **74**, 1823 (1979).
22. A. Hezarkhani, A. E. Williams-Jones, C. H. Gammons, *Miner. Depos.* **34**, 770 (1999).
23. J. M. Proffett, *Econ. Geol.* **98**, 1535 (2003).
24. L. M. Cathles, R. Shannon, *Earth Planet. Sci. Lett.* **262**, 92 (2007).
25. A. von Quadt et al., *Geology* **39**, 731 (2011).
26. J. W. Hedenquist, M. Aoki, H. Shinohara, *Geology* **22**, 585 (1994).
27. R. W. Henley, A. J. Ellis, *Earth Sci. Rev.* **19**, 1 (1983).
28. E. Izawa et al., *J. Geochem. Explor.* **36**, 1 (1990).
29. H. Shinohara, *Rev. Geophys.* **46**, RG4005 (2008).
30. K. F. Evans, A. Genter, J. Sausse, *J. Geophys. Res.* **110**, B04204 (2005).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1225009/DC1
Materials and Methods

Figs. S1 to S3

References and Notes (31–52)

Movies S1 and S2

Database S1

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Apatite $^4\text{He}/^3\text{He}$ and (U-Th)/He Evidence for an Ancient Grand Canyon

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The Grand Canyon is one of the most dramatic features on Earth, yet when and why it was carved have been controversial topics for more than 150 years. Here, we present apatite $^4\text{He}/^3\text{He}$ thermochronometry data from the Grand Canyon basement that tightly constrain the near-surface cooling history associated with canyon incision. $^4\text{He}/^3\text{He}$ spectra for eastern Grand Canyon apatites of differing He date, radiation damage, and U-Th zonation yield a self-consistent cooling history that substantially validates the He diffusion kinetic model applied here. Similar data for the western Grand Canyon provide evidence that it was excavated to within a few hundred meters of modern depths by ~70 million years ago (Ma), in contrast to the conventional model in which the entire canyon was carved since 5 to 6 Ma.

The very existence of the Grand Canyon (Arizona, United States) (Fig. 1) inspires questions about why rivers sometimes carve canyons, how drainage systems and land-

scapes evolve, and how these processes relate to continental elevation gain. The prevailing view is that canyon carving occurred after 5 to 6 million years ago (Ma), when detritus derived from the upstream reaches of the Colorado River system first appeared in Grand Wash Trough at the river's western exit from the Colorado Plateau (1–3). Many consider the absence of such diagnostic deposits before 6 Ma as evidence that the Grand Canyon was not yet excavated (4, 5), with most recent debate focused on how river

integration occurred (5–7). This interpretation assumes that establishment of the integrated Colorado River drainage requires coeval canyon carving.

However, a puzzling array of data hints that the canyon's origin is more complex and could predate integration. Direct geochronologic constraints demanding post-6 Ma formation of the entire canyon do not exist. Dated volcanic rocks drape the western Grand Canyon ≤75 m above modern river level (8), constraining only the most recent ~8% of the total ~1000 m of canyon incision at this location. For the eastern canyon, dated basalts, travertines, and alluvium are ≤0.5 Ma and <200 m above river level (4, 9–11) and resolve <20% of total incision. Speleothem dates may extend this record (12), but their interpretation as incision constraints is debated because it relies on unproven paleohydraulic assumptions (4, 13). A western canyon speleothem date 290 m above river level suggests that the lower ~30% of western canyon carving occurred after ~3.9 Ma (12). Thus, the upper ~70% of the western Grand Canyon lacks any direct geochronologic constraint on when it was carved. In the eastern part of the canyon, 2.19- to 3.72-Ma speleothems located ~900 m above the river (12) imply that the majority of the 1500 m

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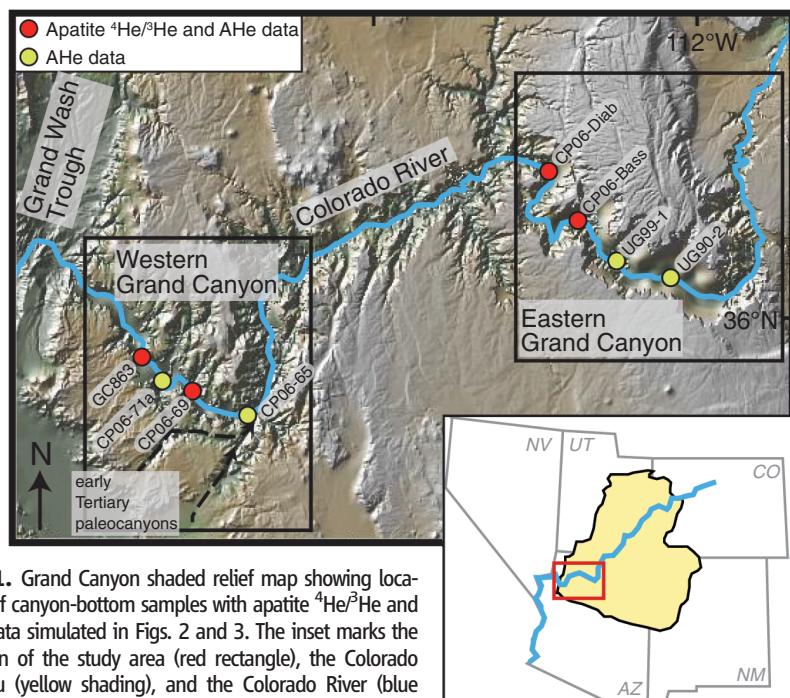


Fig. 1. Grand Canyon shaded relief map showing locations of canyon-bottom samples with apatite $^4\text{He}/^3\text{He}$ and AHe data simulated in Figs. 2 and 3. The inset marks the location of the study area (red rectangle), the Colorado Plateau (yellow shading), and the Colorado River (blue line) in the southwestern United States.

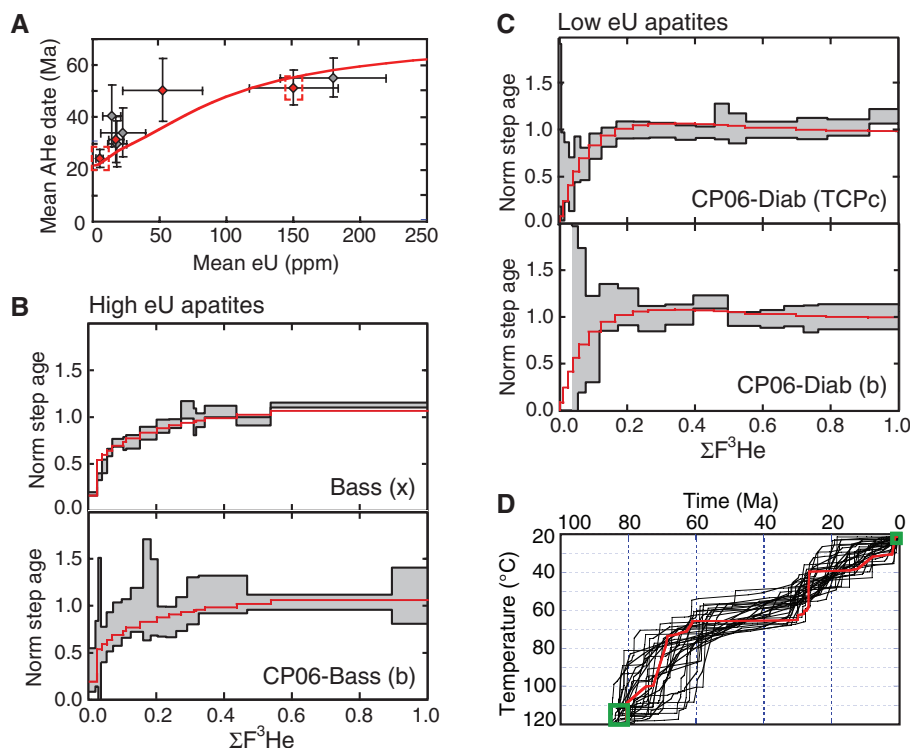


Fig. 2. Results for the eastern Grand Canyon. **(A)** Mean sample AHe date versus mean apatite eU for eight samples (errors at $\pm 1\sigma$ SD). Dashed red boxes mark the two samples with apatite $^4\text{He}/^3\text{He}$ data. Samples with apatite U-Th zonation data are indicated with diamonds, with their AHe dates corrected for α ejection using the mean FTZ (26) values of grains with U-Th zonation data. The red curve shows the predicted date-eU correlation from best-fit thermal history in (D). ppm, parts per million. Normalized $^4\text{He}/^3\text{He}$ step age plots for **(B)** two high eU apatites and **(C)** two low eU apatites, with 1σ uncertainties. Red curves are profiles predicted by best-fit thermal history in (D). $\Sigma F^3\text{He}$, cumulative ^3He release fraction. **(D)** Thermal histories that satisfy the AHe dates for the four samples of variable eU marked by red diamonds in (A) [goodness-of-fit parameter $G = 0.3$ (30)] and the four normalized step age profiles in (B) and (C) ($G = 0.15$). The red line denotes best-fit thermal history. Green boxes are thermal history constraints. Although we have AHe data for eight eastern canyon-bottom samples (23), for clarity only the locations of the four simulated samples are shown in Fig. 1.

of incision at this site occurred after 6 Ma, again subject to paleo-groundwater table assumptions.

Other observations imply an older origin for at least parts of the canyon. Deeply incised paleochannels on the Colorado Plateau's southwestern edge support an extensive northeastward-flowing paleodrainage system that included portions of a paleo-Grand Canyon in the early Tertiary (14–17). Substantial canyon incision between 17 and 6 Ma was inferred from 19-Ma lavas on the plateau surface (15, 18, 19) and from speleothem dates (12), but the latter are controversial, owing to their distal locations from the canyon (13, 20). If an older canyon existed, it is possible that a smaller drainage basin in largely carbonate lithologies explains the absence of pre-6 Ma Colorado River clastics in the Grand Wash Trough (19, 21). Grand Canyon history is further complicated by the possibility that its eastern and western segments evolved independently and later merged into the modern configuration (15). As discussed below, our work supports an east-west dichotomy in incision history, and our data are presented accordingly.

Apatite (U-Th)/He (AHe) thermochronometry can document canyon incision because of its unique sensitivity to topographically induced temperature variations in the shallow crust (22). Rocks cool as they approach Earth's surface by erosion, and AHe data record this cooling history. Prior application of this method to the eastern Grand Canyon suggested incision of a kilometer-scale paleocanyon by 55 Ma, with subsequent downcutting of this canyon below the modern plateau surface in late Tertiary time. This history is compatible with the suggestion that incision of much of the eastern half of the canyon occurred after 6 Ma (23). In contrast, AHe data from the western Grand Canyon suggest excavation to within several hundred meters of the canyon's modern depth by ~ 70 Ma, in direct conflict with the young canyon model (21). The unexpected implications of this initial Grand Canyon AHe work motivated the apatite $^4\text{He}/^3\text{He}$ and U-Th zonation study presented here.

The apatite $^4\text{He}/^3\text{He}$ method provides even greater sensitivity to canyon incision by constraining cooling histories down to $\sim 30^\circ\text{C}$ from the spatial distribution of radiogenic ^4He in the crystal (24). Successful interpretation of both AHe dates and $^4\text{He}/^3\text{He}$ spectra demands accurate understanding of He behavior in apatite. Although the role of radiation damage in retarding apatite He diffusion and the superposition of U-Th zonation effects on $^4\text{He}/^3\text{He}$ spectra have recently been characterized (25–28), verification of the methodology is limited. Because the eastern Grand Canyon yields AHe dates that are generally consistent with previous models of late Tertiary canyon incision, we use this region as a test case for the $^4\text{He}/^3\text{He}$ method. With the use of a recent He diffusion kinetic model (28), our goal is to assess whether the $^4\text{He}/^3\text{He}$ results from this suite—which includes apatites of variable He date, degree of radiation damage,

and U-Th zonation—yield mutually consistent thermal histories. Fulfillment of this expectation validates application of the method to a similar data set from the western Grand Canyon to test the “young” versus “ancient” canyon models.

From two eastern canyon-bottom samples, we acquired $^4\text{He}/^3\text{He}$ spectra on apatites that have a large difference in effective uranium concentration (eU) (29) and mean AHe date (Fig. 2, fig. S1, and tables S1 and S2). We selected these two samples from a suite in which He dates are correlated with eU, diagnostic of the effects of radiation damage on He diffusivity (23) (Fig. 2A). We mapped U and Th concentrations in these apatites plus those from two other samples in the suite (fig. S2 and tables S3 and S4). We performed inverse modeling to find time-temperature paths that simultaneously satisfy the mean AHe dates and the $^4\text{He}/^3\text{He}$ spectra (30) (table S5).

Thermal histories were forced through 110° to 120°C peak temperatures at 80 to 85 Ma, as suggested by complete annealing of apatite fission tracks at this time (31), and cooling to 20° to 25°C surface temperature by present-day. Statistically acceptable paths (30) are characterized by a distinctive two-stage cooling trajectory, impose tight constraints on the ~90° to 30°C thermal history experienced by the eastern gorge, and are consistent with but more restrictive than the history inferred from the AHe dates alone (28) and apatite fission-track (AFT) data from the same area (Fig. 2D) (32). This history records a distinct late Tertiary cooling phase, permissive of substantial post-6 Ma incision. Importantly,

the agreement among samples with differing eU provides compelling evidence that the He diffusion kinetic model we used is appropriate for simulation of Grand Canyon AHe and $^4\text{He}/^3\text{He}$ data.

Given this validation, we examined a similar suite of data from the western Grand Canyon (Fig. 1). Late Cretaceous AHe dates for four canyon-bottom samples (23, 26) show no correlation with apatite eU, consistent with a single-phase cooling history also indicated by AFT data (32) (tables S1 and S6). Apatite $^4\text{He}/^3\text{He}$ data were obtained from two of these samples (Fig. 3, fig. S1, and tables S1 and S2). Duplicate $^4\text{He}/^3\text{He}$ spectra for one sample (CP06-69) are similar, and apatites from this sample are characterized by similar U-Th zonation (fig. S2 and tables S3 and S4). In contrast, the 11 apatite $^4\text{He}/^3\text{He}$ spectra for sample GC863 have diverse shapes, arising from extreme U-Th zoning heterogeneity in this sample (26) (figs. S1 and S2). Because we do not have U-Th zonation data for each apatite with $^4\text{He}/^3\text{He}$ data, this extreme zonation precludes the use of the GC863 $^4\text{He}/^3\text{He}$ results for inverse modeling.

Consequently, we used the $^4\text{He}/^3\text{He}$ spectra from CP06-69 and the AHe dates from all four basement samples (table S5) to constrain statistically acceptable thermal histories for the western Grand Canyon (30). We used the same thermal history constraints as for the eastern Grand Canyon, differing only in broader age bounds of 100 to 80 Ma for the peak temperature, owing to the older AHe dates here. Statistically acceptable paths (30) require rapid cooling to <30°C by

~70 Ma (Fig. 3). Assuming a 20-to-25°C/km geothermal gradient and a 25°C surface temperature (21, 30), this result implies carving of the western Grand Canyon to within several hundred meters of modern depths (70 to 80% of total incision) by 70 Ma. This history is compatible with the volcanic and speleothem data within the western gorge (8, 12).

We used a time-temperature path constructed from a popular description of post-6 Ma incision (4, 30) to explicitly test the young canyon model against our western canyon $^4\text{He}/^3\text{He}$ spectra and bulk AHe dates. The predicted distribution of AHe dates is much broader and includes dates younger than observed (Fig. 3). Similarly, the fits of the predicted $^4\text{He}/^3\text{He}$ spectra to the measurements are statistically unacceptable (Fig. 3B). These conclusions are insensitive to reasonable assumptions about the geotherm and surface temperature and to alternative diffusivity parameters (30) (fig. S3A). The young canyon model also yields a qualitatively poorer fit than the ancient canyon model to the $^4\text{He}/^3\text{He}$ spectra of the strongly eU-zoned sample GC863 (30) (fig. S3B).

The western Grand Canyon $^4\text{He}/^3\text{He}$ and AHe data demand a substantial cooling event at 70 to 80 Ma and provide no evidence for the strong post-6 Ma cooling signal predicted by the young canyon model. Thus, when applying our best understanding of apatite He diffusion kinetics derived from recent work (25, 28), apatite He data support carving of most of the western Grand Canyon by ~70 Ma and are inconsistent with the conventional view that the entire canyon was cut after 6 Ma (4). Moreover, the results imply a dichotomy in eastern and western canyon carving, characterized by coeval excavation of an eastern paleocanyon (23) and substantial carving of the modern western gorge by 70 Ma (21), followed by substantial late Tertiary incision restricted to the eastern canyon. This history supports a model (21) in which much of the Grand Canyon was carved by an ancient Cretaceous river that flowed eastward from western highlands, with Tertiary reversal of the river's course as topography rose in the east and collapsed in the west. Thus, this incision history has profound implications for the evolution of topography, landscapes, hydrology, and tectonism in the North American Cordillera.

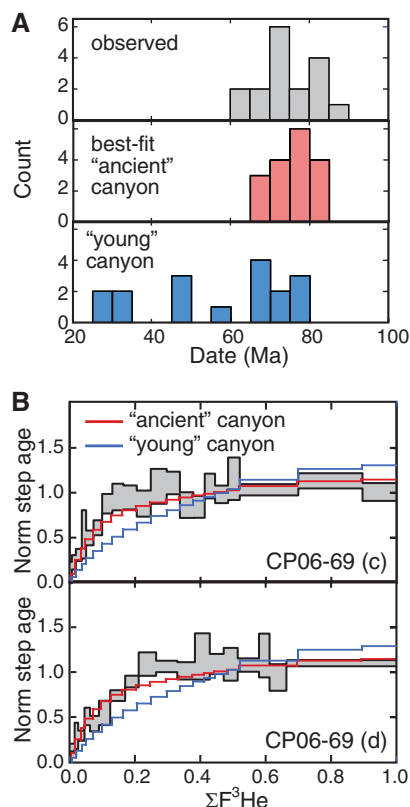


Fig. 3. Results for the western Grand Canyon. **(A)** Distribution of measured AHe dates for multiple replicates of four samples (upper panel) compared with AHe dates predicted by the best-fit thermal history from the inverse modeling suggesting an “ancient” Late Cretaceous canyon [middle histogram, using the red cooling path in (C)], and those predicted by the conventional “young” post-6 Ma canyon model [bottom histogram, using the blue path in (C)]. **(B)** Normalized $^4\text{He}/^3\text{He}$ step age plots for two apatites from CP06-69. Red and blue curves are profiles predicted by the best-fit and young canyon thermal histories in (C), respectively. **(C)** Thermal histories that satisfy the bulk AHe data in (A) ($G = 0.3$) and the two normalized step age profiles in (B) ($G = 0.32$). The red line denotes best-fit thermal history. The blue line shows conventional post-6 Ma incision history based on (4) as described in (30) and yields a substantially poorer fit to the AHe data and $^4\text{He}/^3\text{He}$ spectra in (A) and (B) ($G < 0.06$). Green boxes are thermal history constraints.

References and Notes

1. E. Blackwelder, *Geol. Soc. Am. Bull.* **45**, 551 (1934).
2. C. R. Longwell, *Am. J. Sci.* **244**, 817 (1946).
3. I. Lucchitta, *Geol. Soc. Am. Bull.* **83**, 1933 (1972).
4. K. E. Karlstrom, R. Crow, L. J. Crossey, D. Coblenz, J. W. Van Wijk, *Geology* **36**, 835 (2008).
5. J. L. Pederson, *GSA Today* **18**, 4 (2008).
6. J. E. Spencer, P. A. Pearthree, in *Colorado River Origin and Evolution*, R. A. Young, E. E. Spamer, Eds. (Grand Canyon Association, Grand Canyon, AZ, 2001), pp. 215–222.
7. I. Lucchitta, R. F. Holm, B. K. Lucchitta, *GSA Today* **21**, 4 (2011).
8. K. E. Karlstrom et al., *Geol. Soc. Am. Bull.* **119**, 1283 (2007).
9. J. Pederson, K. Karlstrom, W. Sharp, W. McIntosh, *Geology* **30**, 739 (2002).
10. S. W. Davis et al., in *Colorado River Origin and Evolution*, R. A. Young, E. E. Spamer, Eds. (Grand Canyon Association, Grand Canyon, AZ, 2001), pp. 135–139.

11. I. Lucchitta, G. H. Curtis, M. E. Davis, S. W. Davis, B. Turrin, *Quat. Res.* **53**, 23 (2000).
12. V. Polyak, C. Hill, Y. Asmerom, *Science* **319**, 1377 (2008).
13. J. Pederson, R. Young, I. Lucchitta, L. S. Beard, G. Billingsley, *Science* **321**, 1634b (2008).
14. R. A. Young, *Tectonophysics* **61**, 25 (1979).
15. D. P. Elston, R. A. Young, *J. Geophys. Res.* **96**, 12389 (1991).
16. A. R. Potochnik, in *Colorado River Origin and Evolution*, R. A. Young, E. E. Spamer, Eds. (Grand Canyon Association, Grand Canyon, AZ, 2001), pp. 17–22.
17. R. A. Young, in *Colorado River Origin and Evolution*, R. A. Young, E. E. Spamer, Eds. (Grand Canyon Association, Grand Canyon, AZ, 2001), pp. 7–16.
18. R. A. Young, in *Geology of Grand Canyon, Northern Arizona*, D. P. Elston, G. H. Billingsley, R. A. Young, Eds. (28th International Geological Congress Fieldtrip Guidebook T115/315, American Geophysical Union, Washington, DC, 1989), pp. 166–173.
19. R. A. Young, in *Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Basin: Geologic and Biologic Perspectives*, M. C. Reheis, R. Hershler, D. M. Miller, Eds. (Geological Society of America Special Paper, Geological Society of America, Boulder, CO, 2008), vol. 439, pp. 319–333.
20. P. A. Pearthree, J. E. Spencer, J. E. Faulds, P. K. House, *Science* **321**, 1634 (2008).
21. B. Wernicke, *Geol. Soc. Am. Bull.* **123**, 1288 (2011).
22. M. A. House, B. P. Wernicke, K. A. Farley, *Nature* **396**, 66 (1998).
23. R. M. Flowers, B. P. Wernicke, K. A. Farley, *Geol. Soc. Am. Bull.* **120**, 571 (2008).
24. D. L. Shuster, K. A. Farley, *Earth Planet. Sci. Lett.* **217**, 1 (2004).
25. K. A. Farley, D. L. Shuster, E. B. Watson, K. H. Wanser, G. Balco, *Geochim. Geophys. Geosyst.* **11**, Q10001 (2010).
26. K. A. Farley, D. L. Shuster, R. A. Ketcham, *Geochim. Cosmochim. Acta* **75**, 4515 (2011).
27. D. L. Shuster, R. M. Flowers, K. A. Farley, *Earth Planet. Sci. Lett.* **249**, 148 (2006).
28. R. M. Flowers, R. A. Ketcham, D. L. Shuster, K. A. Farley, *Geochim. Cosmochim. Acta* **73**, 2347 (2009).
29. R. M. Flowers, D. L. Shuster, B. P. Wernicke, K. A. Farley, *Geology* **35**, 447 (2007).
30. Materials and methods are available as supplementary materials on *Science* Online.
31. T. A. Dumitru, I. R. Duddy, P. F. Green, *Geology* **22**, 499 (1994).
32. S. A. Kelley, C. E. Chapin, K. Karlstrom, in *Colorado River Origin and Evolution*, R. A. Young, E. E. Spamer, Eds. (Grand Canyon Association, Grand Canyon, AZ, 2001), pp. 37–42.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1229390/DC1
Materials and Methods
Figs. S1 to S3
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References (33–40)

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Multiplex Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders

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Exome sequencing studies of autism spectrum disorders (ASDs) have identified many de novo mutations but few recurrently disrupted genes. We therefore developed a modified molecular inversion probe method enabling ultra-low-cost candidate gene resequencing in very large cohorts. To demonstrate the power of this approach, we captured and sequenced 44 candidate genes in 2446 ASD probands. We discovered 27 de novo events in 16 genes, 59% of which are predicted to truncate proteins or disrupt splicing. We estimate that recurrent disruptive mutations in six genes—*CHD8*, *DYRK1A*, *GRIN2B*, *TBR1*, *PTEN*, and *TBL1XR1*—may contribute to 1% of sporadic ASDs. Our data support associations between specific genes and reciprocal subphenotypes (*CHD8*-macrocephaly and *DYRK1A*-microcephaly) and replicate the importance of a β -catenin–chromatin-remodeling network to ASD etiology.

There is considerable interest in the contribution of rare variants and de novo mutations to the genetic basis of complex phenotypes such as autism spectrum disorders (ASDs). However, because of extreme genetic heterogeneity, the sample sizes required to implicate any single gene in a complex phenotype are extremely large (*1*). Exome sequencing has

identified hundreds of ASD candidate genes on the basis of de novo mutations observed in the affected offspring of unaffected parents (*2–6*). Yet, only a single mutation was observed in nearly all such genes, and sequencing of over 900 trios was insufficient to establish mutations at any single gene as definitive genetic risk factors (*2–6*).

To address this, we sought to evaluate candidate genes identified by exome sequencing (*2, 3*) for de novo mutations in a much larger ASD cohort. We developed a modified molecular inversion probe (MIP) strategy (Fig. 1A) (*7–9*) with novel algorithms for MIP design; an optimized, automatable work flow with robust performance and minimal DNA input; extensive multiplexing of samples while sequencing; and reagent costs of less than \$1 per gene per sample. Extensive validation using several probe sets and sample collections demonstrated 99% sensitivity and 98%

positive predictive value for single-nucleotide variants at well-covered positions, i.e., 92 to 98% of targeted bases (figs. S1 to S7 and tables S1 to S9) (*10*).

We applied this method to 2494 ASD probands from the Simons Simplex Collection (SSC) (*11*) using two probe sets [ASD1 (6 genes) and ASD2 (38 genes)] to target 44 ASD candidate genes (*12*). Preliminary results using ASD1 on a subset of the SSC implicated *GRIN2B* as a risk locus (*3*). The 44 genes were selected from 192 candidates (*2, 3*) by focusing on genes with disruptive mutations, associations with syndromic autism (*13*), overlap with known or suspected neurodevelopmental copy number variation (CNV) risk loci (*13, 14*), structural similarities, and/or neuronal expression (table S3). Although a few of the 44 genes have been reported to be disrupted in individuals with neurodevelopmental or neuropsychiatric disorders (often including concurrent dysmorphologies), their role in so-called idiopathic ASDs has not been rigorously established. Twenty-three of the 44 genes intersect a 49-member β -catenin–chromatin-remodeling protein-protein interaction (PPI) network (*2*) or an expanded 74-member network (figs. S8 and S9) (*3, 4*).

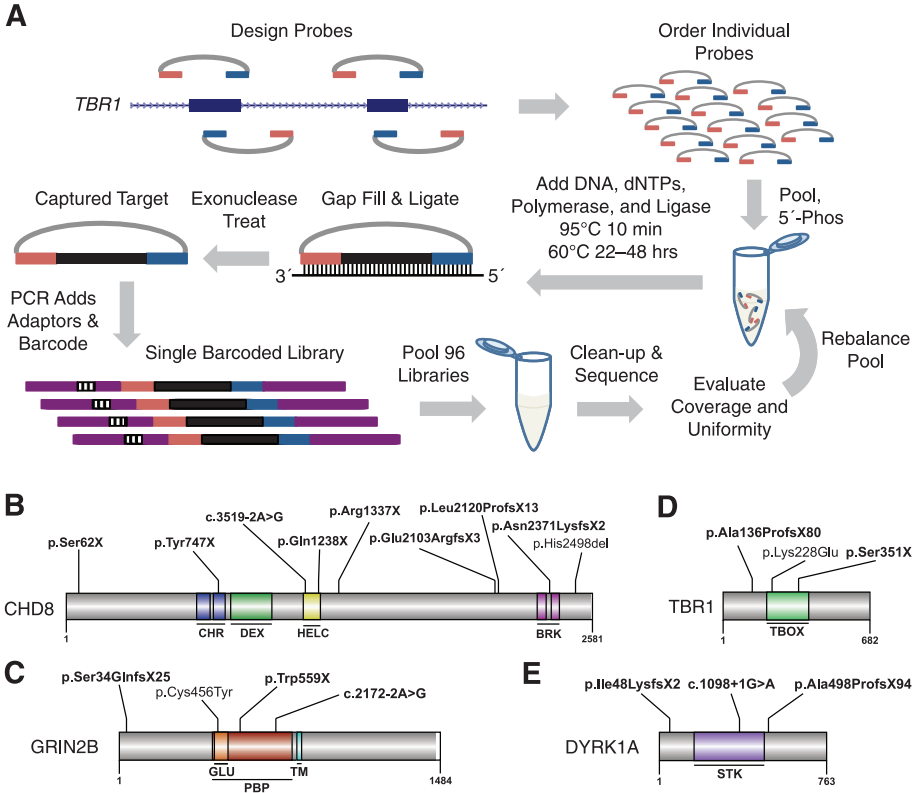
We required samples to successfully capture with both probe sets, yielding 2446 ASD probands with MIP data, 2364 of which had only MIP data and for 82 of which we had also sequenced their exomes (*2, 3*). The high GC content of several candidates required considerable rebalancing to improve capture uniformity (*12*) (figs. S3A and S10). Nevertheless, the reproducible behavior of most MIPs allowed us to identify copy number variation at targeted genes, including several inherited duplications (figs. S11 and S12 and table S10).

To discover de novo mutations, we first identified candidate sites by filtering against variants observed in other cohorts, including non-ASD exomes and MIP-based resequencing of 762 healthy, non-ASD individuals (*12*). The remaining

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Fig. 1. Massively multiplex targeted sequencing identifies recurrently mutated genes in ASD probands. **(A)** Schematic showing design and general work flow of a modified MIP method enabling ultra-low-cost candidate gene resequencing in very large cohorts (figs. S1 to S7 and tables S1 to S9) (10). **(B to E)** Protein diagrams of four genes with multiple de novo mutation events. Significant protein domains for the largest protein isoform are shown (colored regions) as defined by SMART (23) with mutation locations indicated. **(B)** CHD8. **(C)** GRIN2B. **(D)** TBR1. **(E)** DYRK1A. Bold variants are nonsense, frameshifting indels or at splice sites (intron-exon junction is indicated). Domain abbreviations: CHR, chromatin organization modifier; DEX, DEAD-like helicases superfamily; HELC, helicase superfamily C-terminal; BRK, domain in transcription and CHROMO domain helicases; GLU, ligated ion channel L-glutamate- and glycine-binding site; PBP, eukaryotic homologs of bacterial periplasmic substrate binding proteins; TM, transmembrane; STK, serine-threonine kinase catalytic; TBOX, T-box DNA binding.



candidates were further tested by MIP-based resequencing of the proband's parents and, if potentially de novo, confirmed by Sanger sequencing of the parent-child trio (10, 12). We discovered 27 de novo mutations that occurred in 16 of the 44 genes (Fig. 1, B to E; Table 1; and table S11). Consistent with an increased sensitivity for MIP-based resequencing, six of these were not reported in exome-sequenced individuals (Table 1, tables S5 and S11, and fig. S13) (3, 4, 6). Notably, the proportion of de novo events that are severely disruptive, i.e., coding indels, nonsense mutations, and splice-site disruptions (17/27 or 0.63), is four times the expected proportion for random de novo mutations (0.16, binomial $P = 4.9 \times 10^{-8}$) (table S12) (15).

Given their extremely low frequency, accurately establishing expectation for de novo mutations in a locus-specific manner through the sequencing of control trios is impractical. We therefore developed a probabilistic model that incorporates several factors: the overall rate of mutation in coding sequences, estimates of relative locus-specific rates based on human-chimpanzee fixed differences (fig. S14 and table S13), and other factors that may influence the distribution of mutation classes, e.g., codon structure (12). We applied this model to estimate (by simulation) the probability of observing additional de novo mutations during MIP-based resequencing of the SSC cohort. To compare expectation and observation, we treated missense mutations as one class and severe disruptions as a second class. Thus, we could evaluate the probability at a given

Table 1. Six genes with recurrent de novo mutations. Assay is the primary assay that identified the variant. Abbreviations: M, male; F, female; Mut, mutation type; Fs, frameshifting indel; Ns, nonsense; Sp, splice site; Aa, single-amino acid deletion; Ms, missense; EX, exome; HGVS, Human Genome Variation Society nomenclature; NVIQ, nonverbal intellectual quotient.

Proband	Sex	Gene	Mut	Assay	HGVS	NVIQ
12714.p1	M	CHD8*	Ns	MIP	p.Ser62X	78
13986.p1	M	CHD8*	Fs	MIP	p.Tyr747X	38
11654.p1	F	CHD8*	Sp	MIP† (4)	c.3519-2A>G	41
13844.p1	M	CHD8*	Ns	EX	p.Gln1238X	34
14016.p1	M	CHD8*	Ns	MIP	p.Arg1337X	92
12991.p1	M	CHD8*	Fs	MIP	p.Glu2103ArgfsX3	67
12752.p1	F	CHD8*	Fs	EX	p.Leu2120ProfsX13	93
14233.p1	M	CHD8*	Fs	MIP	p.Asn2371LysfsX2	19
14406.p1	M	CHD8*	Aa	MIP	p.His2498del	98
12099.p1	M	DYRK1A*	Fs	MIP† (4)	p.Ile48LysfsX2	55
13890.p1	F	DYRK1A*	Sp	EX	c.1098+1G>A	42
13552.p1	M	DYRK1A*	Fs	MIP‡ (6)	p.Ala498ProfsX94	66
11691.p1	M	GRIN2B†	Fs	MIP‡ (3)	p.Ser34GlnfsX25	62
13932.p1	M	GRIN2B†	Ms	MIP	p.Cys456Tyr	55
12547.p1	M	GRIN2B†	Ns	MIP	p.Trp559X	65
12681.p1	F	GRIN2B†	Sp	EX	c.2172-2A>G	65
14433.p1	M	PTEN	Ms	MIP	p.Thr131Ile	50
14611.p1	M	PTEN	Fs	MIP	p.Cys136MetfsX44	33
11390.p1	F	PTEN	Ms	EX	p.Thr167Asn	77
12335.p1	F	TBL1XR1*	Ms	EX	p.Leu282Pro	47
14612.p1	M	TBL1XR1*	Fs	MIP	p.Ile397SerfsX19	41
11480.p1	M	TBR1†	Fs	EX	p.Ala136ProfsX80	41
13814.p1	M	TBR1†	Ms	MIP	p.Lys228Glu	78
13796.p1	F	TBR1†	Fs	MIP† (4)	p.Ser351X	63

*Part of 49-member connected component reported in (3). †Part of expanded 74-member connected component. ‡,§Proband was exome sequenced by cited study and variant was ‡not reported or §reported. ||Variant reported in MIP screen from (3).

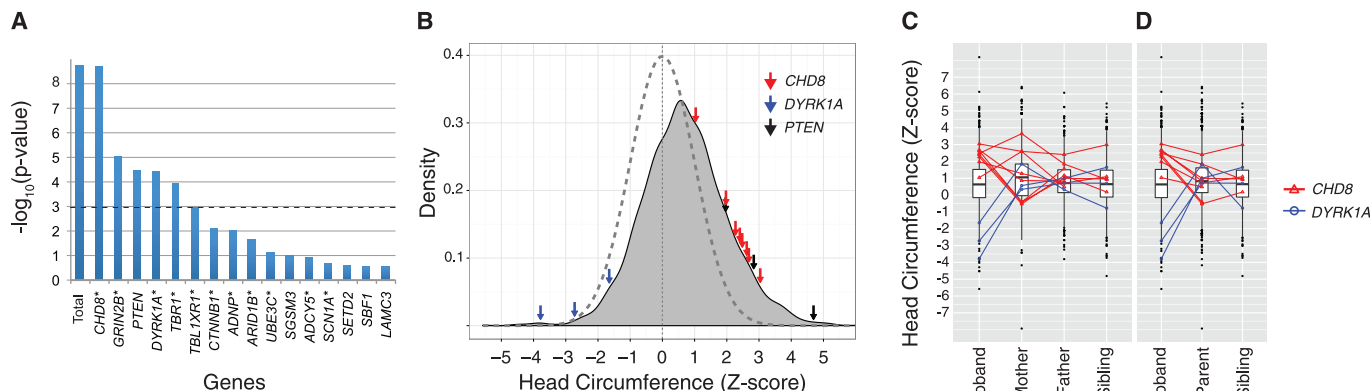


Fig. 2. Locus-specific mutation probabilities and associated phenotypes. **(A)** Estimated P values for the observed number of additional de novo mutations identified in the MIP screen of 44 ASD candidate genes. Probabilities shown are for observing x or more events, of which at least y belong to the severe class. The observed numbers of mutations in all 44 genes ("Total") and *CHD8* were not seen in any of 5×10^8 simulations. Based on the simulation mean (0.0153), the Poisson probability for seven or more severe class *CHD8* mutations is 3.8×10^{-17} . Dashed line Bonferroni corrected significance threshold

for $\alpha = 0.05$. *Gene product in the 74-member PPI connected component. **(B to D)** Standardized head circumference (HC) Z scores for SSC. **(B)** All probands screened with superimposed normal distribution (dashed). HC Z scores for individuals with de novo truncating and/or splice mutations highlighted for *CHD8* (red arrows), *DYRK1A* (blue arrows), and *PTEN* (black arrows). **(C and D)** Box and whisker plots of the HC Z scores for the SSC. Mutations carriers are shown and linked to their respective family members. **(C)** All family members. **(D)** Only proband sex-matched family members.

locus of observing at least x de novo mutations, of which at least y belong to the severe class.

We found evidence of mutation burden—a higher rate of de novo mutation than expected—in the overall set of 44 genes (observed $n = 27$ versus mean expected $n = 5.6$, simulated $P < 2 \times 10^{-9}$) (Fig. 2A). The burden was driven by the severe class (observed $n = 17$ versus mean expected $n = 0.58$, simulated $P < 2 \times 10^{-9}$). Most severe class mutations intersected the 74-member PPI network (16 out of 17), although only 23 out of 44 genes are in this network (binomial $P = 0.0002$) (12). Furthermore, 21 out of 27 mutations occurred in network-associated genes (binomial $P = 0.004$). Of the six individual genes (*CHD8*, *GRIN2B*, *DYRK1A*, *PTEN*, *TBR1*, and *TBL1XR1*) with evidence of mutation burden [alpha of 0.05 after a Holm-Bonferroni correction for multiple testing (Fig. 2A); *TBL1XR1* is borderline significant with a more conservative Bonferroni correction], five fall within the β -catenin-chromatin-remodeling network. In our combined MIP and exome data, ~1% (24 out of 2573) of ASD probands harbor a de novo mutation in one of these six genes, with *CHD8* representing 0.35% (9 out of 2573) (Fig. 1B and Table 1).

For these analyses, we conservatively used the highest available empirical estimate of the overall mutation rate in coding sequences (3). With the exception of *TBL1XR1*, these results were robust to doubling the overall mutation rate or to using the upper bound of the 95% confidence interval of the locus-specific rate estimate for each of these genes (10). Moreover, we obtained similar results regardless of whether parameters were estimated from rare, segregating variation or from de novo mutations in unaffected siblings (10), as well as with a sequence composition model based on genome-wide de novo mutation (16). Exome sequencing of non-ASD individuals (unaffected

siblings or non-ASD cohorts) further supports these conclusions (table S14) (10).

We also validated 23 inherited, severely disruptive variants in the 44 genes (table S15). Two probands with such variants carry de novo 16p11.2 duplications (table S16). Combining de novo and inherited events, severe class variants were observed at twice the rate in MIP-sequenced probands as compared with MIP-sequenced healthy, non-ASD individuals (Fisher's exact test, $P = 0.083$). Severe class variants were not transmitted to 14 out of 20 unaffected siblings (binomial $P = 0.058$) (table S15). However, larger cohorts than currently exist will be needed to fully evaluate these modest trends.

We analyzed phenotypic data on probands with mutations in the six implicated genes. Each was diagnosed with autism on the basis of current, strict, gold-standard criteria. No obvious dysmorphologies or recurrent comorbidities were present. Probands tended to fall into the intellectual disability range for nonverbal IQ (NVIQ) (mean 58.3) (Table 1). However, for *CHD8*, probands were found to have NVIQ scores ranging from profoundly impaired to average (mean 62.2, range 19 to 98).

Given the previously observed microcephaly in our index *DYRK1A* mutation case, macrocephaly in both probands with *CHD8* mutations (3), and the association of these traits with other syndromic loci (13, 17), we reexamined head circumference (HC) in the larger set of probands with protein-truncation or splice-site de novo events using age- and sex-normalized HC Z scores (12) (Fig. 2B). For *CHD8* ($n = 8$), we observed significantly larger head sizes relative to individuals screened without *CHD8* mutations (two-sample permutation test, two-sided $P = 0.0007$). De novo *CHD8* mutations are present in ~2% of macrocephalic (HC > 2.0) SSC probands ($n = 366$,

which suggests a useful phenotype for patient subclassification. For *DYRK1A* ($n = 3$), we observed significantly smaller head sizes relative to individuals screened without *DYRK1A* mutations (two-sample permutation test, two-sided $P = 0.0005$). Comparison of head size in the context of the families (Fig. 2, C and D, and table S17) provides further support for this reciprocal trend (10). These findings are also consistent with case reports of patients with structural rearrangements and mouse transgenic models that implicate *DYRK1A* and *CHD8* as regulators of brain growth (18–21). Macrocephaly was also observed in individuals with de novo and inherited *PTEN* mutations (22).

Our data support an important role for de novo mutations in six genes in ~1% of sporadic ASDs. As the SSC was specifically established for simplex families and as its probands generally have higher cognitive functioning than has been reported in other ASD cohorts (11), it is unknown how our findings will translate into other cohorts. Furthermore, whereas our data implicate specific loci in ASDs, they are insufficient to evaluate whether the observed de novo mutations are sufficient to cause ASDs (tables S16 and S18).

Exome sequencing and CNV studies suggest that there are hundreds of relevant genetic loci for ASDs. Technologies and study designs directed at identifying de novo mutations, both for the discovery of ASD candidate genes, as well as for their validation, provide sufficient power to implicate individual genes from a relatively small number of events. The analytical framework described here can be applied to any other disorder—simple or complex—for which de novo coding mutations are suspected to contribute to risk. In addition, the experimental methods presented here are broadly useful for the rapid and economical resequencing of candidate

genes in extremely large cohorts, as may be required for the definitive implication of rare variants or de novo mutations in any genetically complex disorder.

References and Notes

- G. V. Kryukov, A. Shpunt, J. A. Stamatiou, S. R. Sunyaev, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3871 (2009).
- B. J. O'Roak *et al.*, *Nat. Genet.* **43**, 585 (2011).
- B. J. O'Roak *et al.*, *Nature* **485**, 246 (2012).
- S. J. Sanders *et al.*, *Nature* **485**, 237 (2012).
- B. M. Neale *et al.*, *Nature* **485**, 242 (2012).
- I. Iossifov *et al.*, *Neuron* **74**, 285 (2012).
- E. H. Turner, C. Lee, S. B. Ng, D. A. Nickerson, J. Shendure, *Nat. Methods* **6**, 315 (2009).
- G. J. Porreca *et al.*, *Nat. Methods* **4**, 931 (2007).
- S. Krishnakumar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 9296 (2008).
- See supplementary text on Science Online.
- G. D. Fischbach, C. Lord, *Neuron* **68**, 192 (2010).
- Materials and methods are available as supplementary materials on Science Online.
- C. Betancur, *Brain Res.* **1380**, 42 (2011).
- G. M. Cooper *et al.*, *Nat. Genet.* **43**, 838 (2011).
- M. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 961 (2010).
- A. Kong *et al.*, *Nature* **488**, 471 (2012).
- C. A. Williams, A. Dagli, A. Battaglia, *Am. J. Med. Genet. A.* **146A**, 2023 (2008).
- R. S. Møller *et al.*, *Am. J. Hum. Genet.* **82**, 1165 (2008).
- B. W. van Bon *et al.*, *Clin. Genet.* **79**, 296 (2011).
- F. Guedj *et al.*, *Neurobiol. Dis.* **46**, 190 (2012).
- M. E. Talkowski *et al.*, *Cell* **149**, 525 (2012).
- J. Zhou, L. F. Parada, *Curr. Opin. Neurobiol.* **22**, 873 (2012).
- I. Letunic, T. Doerks, P. Bork, *Nucleic Acids Res.* **40** (Database issue), D302 (2012).

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Initiative (SFARI) Base. Approved researchers can obtain the SSC population dataset described in this study ([https://ordering.base.sfari.org/~browse_collection/archive\[sscc_v13\]/ui/view](https://ordering.base.sfari.org/~browse_collection/archive[sscc_v13]/ui/view)) by applying at <https://base.sfari.org>. This work was supported by grants from the Simons Foundation (SFARI 137578, 191889 to E.E.E., J.S., and R.B.), NIH HD065285 (E.E.E. and J.S.), NIH NS069605 (H.C.M.), and R01 NS064077 (D.D.). E.B. is an Alfred P. Sloan Research Fellow. E.E.E. is an Investigator of the Howard Hughes Medical Institute. Scientific advisory boards or consulting affiliations: Ariosa Diagnostics (J.S.), Stratos Genomics (J.S.), Good Start Genetics (J.S.), Adaptive Biotechnologies (J.S.), Pacific Biosciences (E.E.E.), Synapse Dx (E.E.E.), DNAnexus (E.E.E.), and SFARI GENE (H.C.M.). B.J.O. is an inventor on patent PCT/US2009/30620: Mutations in contactin associated protein 2 are associated with increased risk for idiopathic autism. Raw sequencing data available at the National Database for Autism Research, NDARCOL1878.

Supplementary Materials

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Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell

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Kindred cells can have different genomes because of dynamic changes in DNA. Single-cell sequencing is needed to characterize these genomic differences but has been hindered by whole-genome amplification bias, resulting in low genome coverage. Here, we report on a new amplification method—multiple annealing and looping-based amplification cycles (MALBAC)—that offers high uniformity across the genome. Sequencing MALBAC-amplified DNA achieves 93% genome coverage $\geq 1\times$ for a single human cell at 25x mean sequencing depth. We detected digitized copy-number variations (CNVs) of a single cancer cell. By sequencing three kindred cells, we were able to identify individual single-nucleotide variations (SNVs), with no false positives detected. We directly measured the genome-wide mutation rate of a cancer cell line and found that purine-pyrimidine exchanges occurred unusually frequently among the newly acquired SNVs.

Single-molecule and single-cell studies reveal behaviors that are hidden in bulk measurements (1, 2). In a human cell, the genetic information is encoded in 46 chromosomes. The variations occurring in these chromosomes, such as single-nucleotide variations (SNVs) and copy-number variations (CNVs) (3), are the driving forces in biological processes such as evo-

lution and cancer. Such dynamic variations are reflected in the genomic heterogeneity among a population of cells, which demands characterization of genomes at the single-cell level (4–6). Single-cell genomics analysis is also necessary when the number of cells available is limited to few or one, such as prenatal testing samples (7, 8), circulating tumor cells (9), and forensic specimens (10).

Prompted by rapid progress in next-generation sequencing techniques (11), there have been several reports on whole-genome sequencing of single cells (12–16). These methods have relied on whole-genome amplification (WGA) of an individual cell to generate enough DNA for sequencing (17–21). However, WGA methods in general are prone to amplification bias, which results in

low genome coverage. Polymerase chain reaction (PCR)-based WGA introduces sequence-dependent bias because of the exponential amplification with random primers (17, 18, 22). Multiple displacement amplification (MDA), which uses random priming and the strand-displacing $\phi 29$ polymerase under isothermal conditions (19), has provided improvements over PCR-based methods but still exhibits considerable bias, again due to nonlinear amplification.

Here we report a new WGA method, multiple annealing and looping-based amplification cycles (MALBAC), which introduces quasilinear preamplification to reduce the bias associated with nonlinear amplification. Picograms of DNA fragments (~10 to 100 kb) from a single human cell serve as templates for amplification with MALBAC (Fig. 1). The amplification is initiated with a pool of random primers, each having a common 27-nucleotide sequence and 8 variable nucleotides that can evenly hybridize to the templates at 0°C. At an elevated temperature of 65°C, DNA polymerases with strand-displacement activity are used to generate semiamplicons with variable lengths (0.5 to 1.5 kb), which are then melted off from the template at 94°C. Amplification of the semiamplicons gives full amplicons that have complementary ends. The temperature is cycled to 58°C to allow the looping of full amplicons, which prevents further amplification and cross-hybridizations. Five cycles of preamplification are followed by exponential amplification of the full amplicons by PCR to generate micrograms of DNA required for next-generation sequencing (Fig. 1). In the PCR, oligonucleotides with the common 27-nucleotide sequence are used as the primers.

We used MALBAC to amplify the DNA of single SW480 cancer cells. With ~25x mean

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Fig. 1. MALBAC single-cell whole-genome amplification. A single cell is picked and lysed. First, genomic DNA of the single cell is melted into single-stranded DNA molecules at 94°C. MALBAC primers then anneal randomly to single-stranded DNA molecules at 0°C and are extended by a polymerase with displacement activity at elevated temperatures, creating semiamplicons. In the following five temperature cycles, after the step of looping the full amplicons, single-stranded amplicons and the genomic DNA are used as a template to produce full amplicons and additional semiamplicons, respectively. For full amplicons, the 3' end is complementary to the sequence on the 5' end. The two ends hybridize to form looped DNA, which can efficiently prevent the full amplicon from being used as a template, therefore warranting a close-to-linear amplification. After the five cycles of linear preamplification, only the full amplicons can be exponentially amplified in the following PCR using the common 27-nucleotide sequence as the primer. PCR reaction will generate microgram level of DNA material for sequencing experiments.

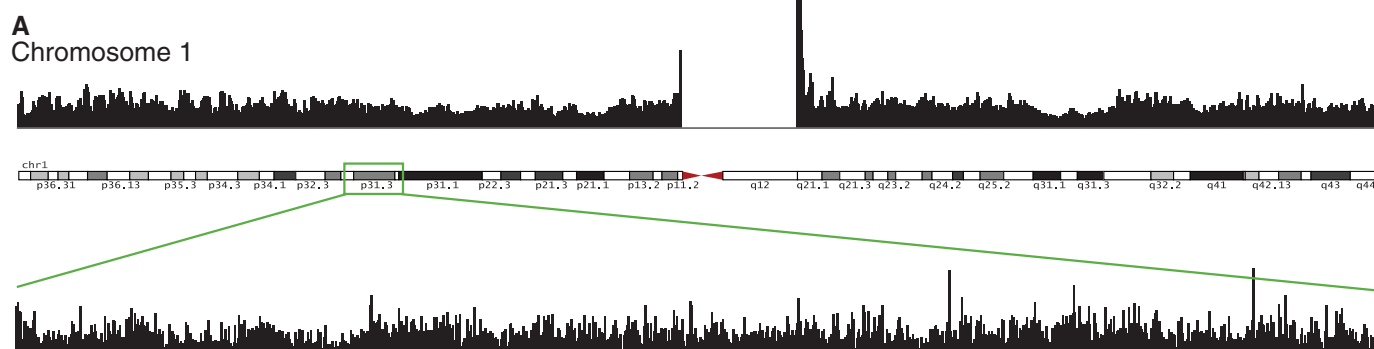
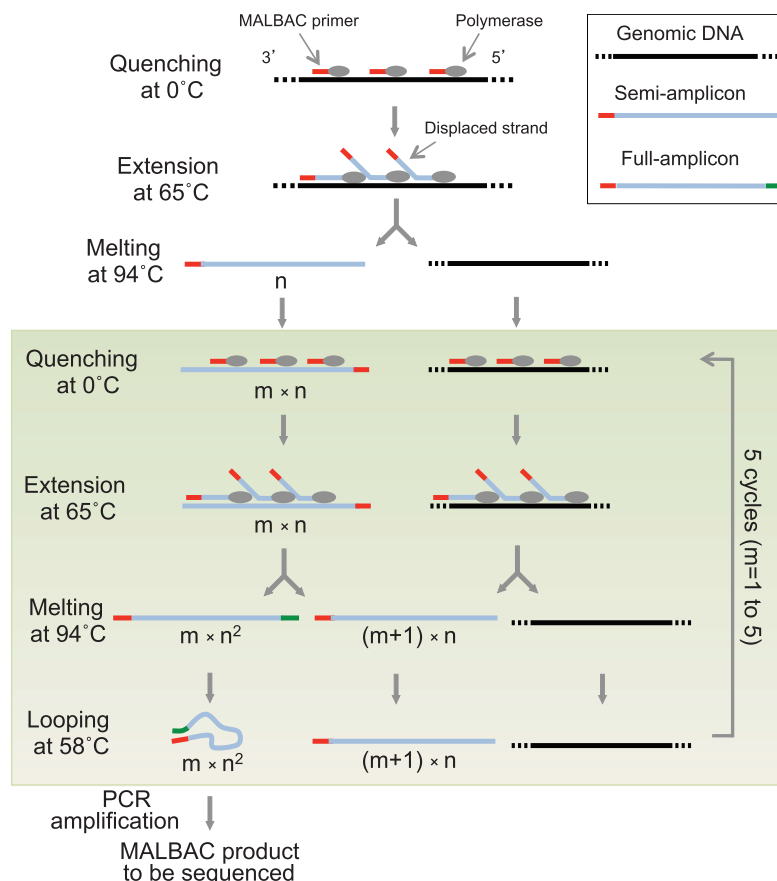


Fig. 2. Characterization of amplification uniformity.

(A) Histograms of reads over the entirety of chromosome 1 (chr1) of a single cell from the SW480 cancer cell line and the zoom-in of an ~8-million-base region (chr1: 62,023,147 to 70,084,845). (B) Lorenz curves of MALBAC, MDA, and the bulk sample. A Lorenz curve gives the cumulative fraction of reads as a function of the cumulative fraction of genome. Perfectly uniform coverage would result in a diagonal line, and a large deviation from the diagonal is indicative of biased coverage. The blue and green arrows indicate the uncovered fractions of the genome for MALBAC and MDA, respectively. All samples are sequenced at 25x depth. (C) Power spectrum of read density throughout the genome (as a function of spatial frequency). MALBAC performs similarly to bulk, whereas the MDA spectrum shows high amplitude at low frequency, demonstrating that regions of several megabases suffer from under- and overamplification. This observation is consistent with the variations of read depth in fig. S3.

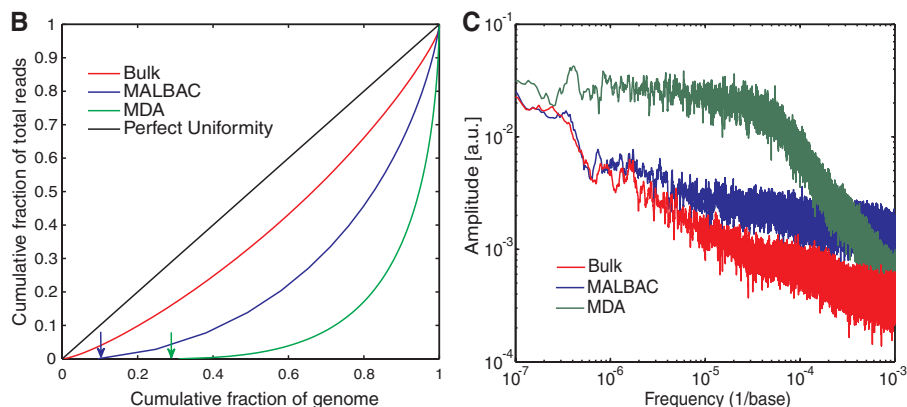
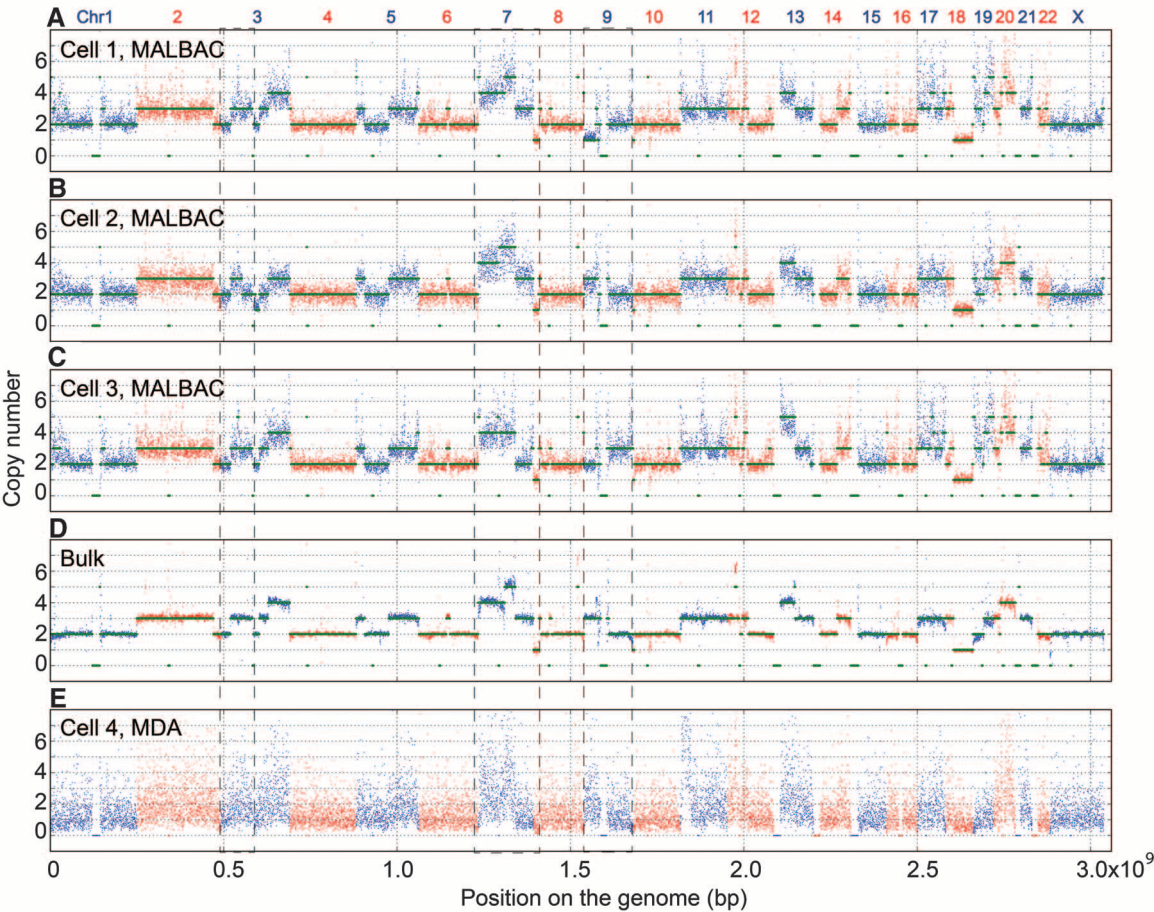


Fig. 3. CNVs of single cancer cells. Digitized copy numbers across the genome are plotted for three single cells (**A** to **C**) as well as the bulk sample (**D**) from the SW480 cancer cell line. The bottom panel shows the result based on MDA amplification (**E**). Green lines are fitted CNV numbers obtained from the hidden Markov model (see supplementary materials). The single cells are sequenced at only 0.8x depth, whereas the bulk and MDA are done at 25x. More single-cell CNV analyses are included in the supplementary materials (fig. S4). The regions within the dashed box exhibit the CNV differences among single cells and the bulk, which cannot be resolved by MDA. The binning window is 200 kb.



sequencing depth, we consistently achieved ~85% and up to 93% genome coverage at $\geq 1\times$ depth on either strand (Fig. 2A). As a comparison, we performed MDA on a single cell from the same cancer cell line. At 25x mean sequencing depth, MDA covered 72% of the genome at $\geq 1\times$ coverage. Although substantial variations of the coverage have been reported for MDA (15, 16, 20, 23), MALBAC coverage is reproducible.

We used Lorenz curves to evaluate coverage uniformity along the genome. We plotted the cumulative fraction of the total reads that cover a given cumulative fraction of genome (Fig. 2B). The diagonal line indicates a perfectly uniform distribution of reads, and deviation from the diagonal line indicates an uneven distribution of reads. We compared the Lorenz curves for bulk sequencing, MALBAC, and MDA at ~25x mean sequencing depth (Fig. 2B). It is evident that MALBAC outperforms MDA in uniformity of genome coverage. We also plotted the power spectrum of read density variations to show the spatial scale at which the variations take place. For MDA, large amplitudes at low frequencies (inverse genome distance) were observed, indicating that large contiguous regions of the genome are over- or underamplified. In contrast, MALBAC has a power spectrum similar to that of the unamplified bulk.

Table 1. Comparison of single-cell SNVs for bulk, MDA, and MALBAC.

	Heterozygous SNVs	Homozygous SNVs	Total SNVs
<i>Bulk</i>			
SNVs	911,958	1,930,204	2,842,162
<i>Single-cell MDA</i>			
SNVs	93,140 (2,828)*	1,238,286 (1,973)	1,331,426 (4,801)
Detection efficiency	10%	63%	41%
<i>Single-cell MALBAC</i>			
SNVs	756,812 (108,481)	1,539,326 (6,821)	2,296,138 (115,302)
Detection efficiency	71%	80%	76%

*The number in parentheses indicates the number of false positives.

Table 2. MALBAC identification of total SNVs and newly acquired SNVs using two and three kindred cells.

	Heterozygous SNVs	Homozygous SNVs	Total SNVs
<i>Two kindred cells</i>			
SNVs	615,387	1,322,555	1,937,942
Detection efficiency	67%	68%	68%
Newly acquired SNVs	145 (~100)*	3 (~0)	148 (~100)
<i>Three kindred cells</i>			
SNVs	660,246	1,577,798	2,238,044
Detection efficiency	72%	81%	80%
Newly acquired SNVs	30 (~0)	5 (~0)	35 (~0)

*The number in parentheses indicates the number of false positives. “~0” indicates undetected in Sanger sequencing when PCR primers can be readily designed.

CNVs due to insertions, deletions, or multiplications of genome segments are frequently observed in almost all categories of human tumors (13, 24, 25). MALBAC's lack of large-scale bias makes it amenable to probing CNVs in single cells. We determined the digitized CNVs

across the whole genomes of three individual cells from the SW480 cancer cell line (Fig. 3, A to C). CNVs of five cells are included in the supplementary materials. The chromosomes exhibit distinct CNV differences among the three individual cancer cells and in the bulk result (Fig.

3D), which are difficult to resolve by MDA (Fig. 3E). For the MALBAC data, we used a hidden Markov model to quantify CNVs (see supplementary materials). We confirmed the gross features of CNVs detected by MALBAC with a previously published karyotyping study (26).

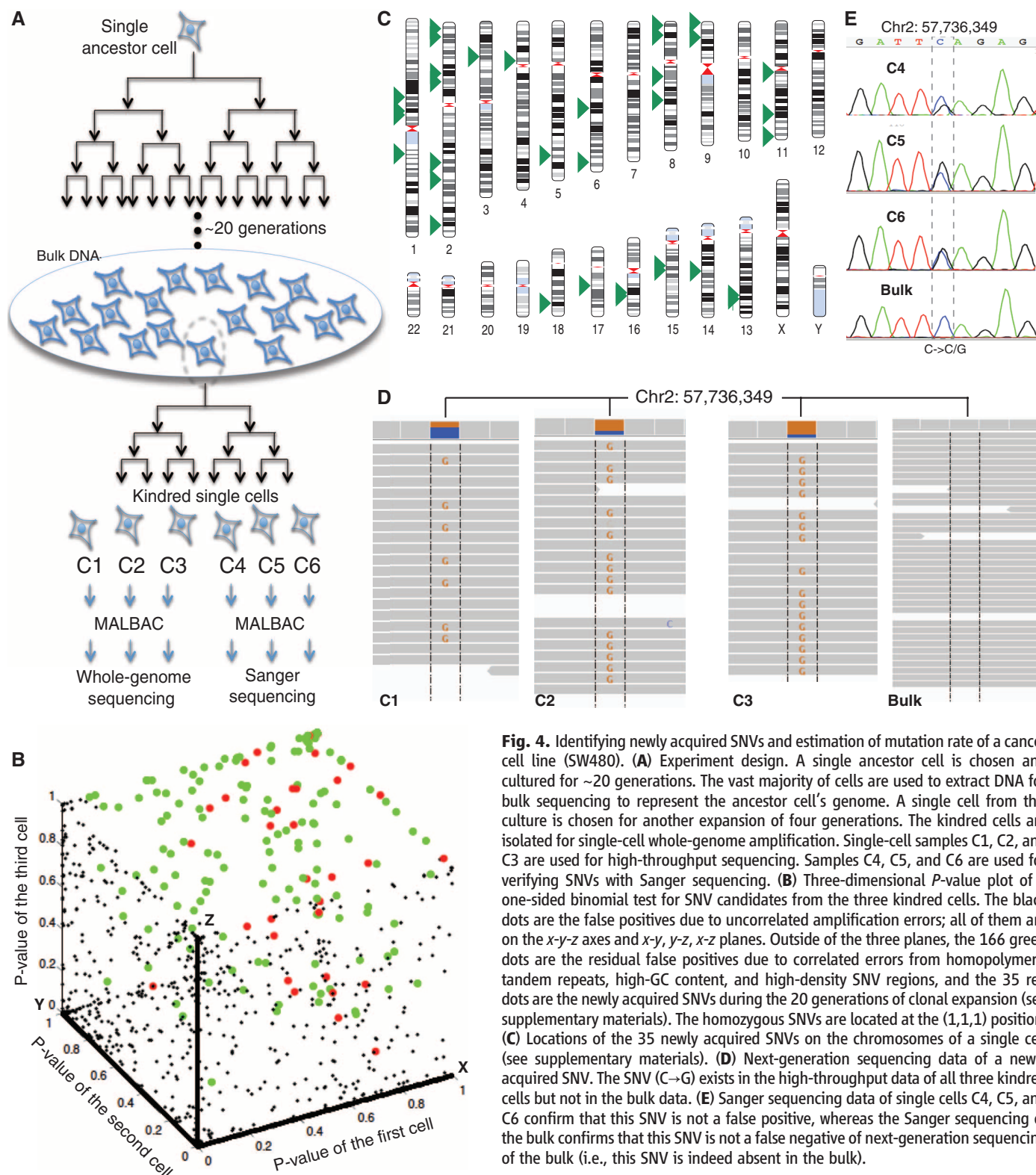


Fig. 4. Identifying newly acquired SNVs and estimation of mutation rate of a cancer cell line (SW480). **(A)** Experiment design. A single ancestor cell is chosen and cultured for ~20 generations. The vast majority of cells are used to extract DNA for bulk sequencing to represent the ancestor cell's genome. A single cell from this culture is chosen for another expansion of four generations. The kindred cells are isolated for single-cell whole-genome amplification. Single-cell samples C1, C2, and C3 are used for high-throughput sequencing. Samples C4, C5, and C6 are used for verifying SNVs with Sanger sequencing. **(B)** Three-dimensional P -value plot of a one-sided binomial test for SNV candidates from the three kindred cells. The black dots are the false positives due to uncorrelated amplification errors; all of them are on the x - y - z axes and x - y , y - z , x - z planes. Outside of the three planes, the 166 green dots are the residual false positives due to correlated errors from homopolymers, tandem repeats, high-GC content, and high-density SNV regions, and the 35 red dots are the newly acquired SNVs during the 20 generations of clonal expansion (see supplementary materials). The homozygous SNVs are located at the (1,1,1) position. **(C)** Locations of the 35 newly acquired SNVs on the chromosomes of a single cell (see supplementary materials). **(D)** Next-generation sequencing data of a newly acquired SNV. The SNV (C→G) exists in the high-throughput data of all three kindred cells but not in the bulk data. **(E)** Sanger sequencing data of single cells C4, C5, and C6 confirm that this SNV is not a false positive, whereas the Sanger sequencing of the bulk confirms that this SNV is not a false negative of next-generation sequencing of the bulk (i.e., this SNV is indeed absent in the bulk).

For example, both MALBAC-based quantification of CNVs and spectral karyotyping show one copy of chromosome 18 and three copies of chromosome 17 in the SW480 cancer cell line. Although the majority of copy numbers are consistent between single cells, we also observe cell-to-cell variations as labeled by the dashed boxes in Fig. 3.

Attempts have been made recently to identify SNVs from a single cell by MDA (15, 16, 23). The first challenge in accurate SNV identification from a single cell is substantial human contamination from the environment and the operators, given picograms of DNA from a single human cell. The second challenge is low detection yield (high false negative rates), particularly where alleles drop out due to amplification bias. The third challenge is false positives associated with amplification and sequencing errors, either random or systematic (27).

To meet the first challenge, we took special precautions to decontaminate with ultraviolet radiation before each experiment was conducted in a restricted clean room. An alternative approach to reduce contamination is microfluidics (28).

With regard to the second challenge, MALBAC allowed us to identify 2.2×10^6 single-cell SNVs compared with 2.8×10^6 detected SNVs in bulk, yielding a 76% detection efficiency, in contrast to 41% with MDA (Table 1). This improvement resulted from improved uniformity by MALBAC (fig. S6). Listed separately in Table 1 are heterozygous and homozygous SNVs. Next, we calculated the allele dropout rate. Comparison of single-cell and bulk SNVs showed that 7288 of the SNVs genotyped as homozygous mutations by MALBAC are actually heterozygous in bulk, which corresponds to a ~1% allele dropout rate in MALBAC (see supplementary materials). In contrast, with MDA we found 172,563 incorrect homozygous identifications, corresponding to an allele dropout rate of ~65% (see supplementary materials).

Compared to the bulk data, the MALBAC data contains 1.1×10^5 false positives (Table 1) out of 3×10^9 bases in the genome. This corresponds to a $\sim 4 \times 10^{-5}$ false-positive rate, which is due to the errors made by the polymerases in the semiamplicons generated in the first MALBAC cycle and propagated through the later amplification. Although improving the polymerase's error rate is possible, our strategy to reduce the false-positive rate was to sequence two or three kindred cells derived from the same cell. The simultaneous appearance of an SNV in the kindred cells would indicate a true SNV. The false-positive rate due to uncorrelated random errors can be reduced to $\sim 10^{-8}$ with two kindred cells and $\sim 10^{-12}$ with three kindred cells.

However, there are false positives due to correlated errors—that is, systematic sequencing and amplification errors. We filtered out these errors by comparing two unrelated single cells that are not from the same lineage (fig. S5) and additional

screening. After this procedure, we can identify true SNVs of a single cell with no false positives detected by Sanger sequencing, as described below (Table 2).

To gain insight into the mutation process in the cancer cells, we clonally expanded a single ancestor cell picked from a heterogeneous population of the SW480 cancer cell line for 20 generations (Fig. 4A). We extracted DNA from this single-cell clonal expansion for bulk sequencing, which reflects the genome of the ancestor cell. We then picked a single cell from this clone. To detect SNVs acquired by the cell during expansion, we grew another four generations to obtain the kindred cells denoted C1 to C16. We individually sequenced three kindred cells—C1, C2, and C3—after MALBAC amplification. After filtering correlated and uncorrelated errors (Fig. 4B), we detected 35 unique SNVs shown in Fig. 4C.

We took 24 out of 35 unique SNVs for which we can readily design PCR primers and confirmed that they are neither false positives by Sanger sequencing C4 to C6 nor false negatives by Sanger sequencing the bulk. (See the supplementary materials for Sanger sequencing data.) As an example, Fig. 4, D and E shows the MALBAC and Sanger sequencing result of one such SNV.

These 35 unique SNVs are newly acquired during the 20 cell divisions. Adjusting for a detection efficiency of 72% for heterozygous SNVs, we estimate that ~49 mutations occurred in the 20 generations, yielding a mutation rate of ~2.5 nucleotides per cell generation, consistent with our estimation based on the bulk data (see supplementary materials). The mutation rate of this cancer cell line is about 10 times as high as the mutation rate estimated based on germline studies (29–31).

Mutations can be transitions (purine↔purine exchange, i.e., A↔G, or pyrimidine↔pyrimidine exchange, i.e., C↔T) or transversions (purine↔pyrimidine exchanges, i.e., A/G↔C/T). Transitions are more common. Unexpectedly, we found that the transition/transversion (tstv) ratio for the 35 newly acquired SNVs detected is only 0.30, whereas the ratio for the total SNVs of this cell line is 2.01, as expected for common human mutations (32). To further confirm that this observation is not due to single-cell amplification, we sequenced the bulk DNA of the original heterogeneous culture (see supplementary materials). The tstv ratio for SNVs detected in the single-cell expanded bulk but not in the original heterogeneous bulk was 0.75. Both significantly low tstv ratios indicate that transitions are not favored over transversion for newly acquired SNVs in this cancer cell line (see supplementary materials). Although understanding the underlying mechanism of this phenomenon will require similar measurements in other systems, it is evident that, by allowing precise characterization of CNVs and SNVs, MALBAC can shed light on the individuality, heterogeneity, and dynamics of the genomes of single cells.

References and Notes

1. M. B. Elowitz, A. J. Levine, E. D. Siggia, P. S. Swain, *Science* **297**, 1183 (2002).
2. G. W. Li, X. S. Xie, *Nature* **475**, 308 (2011).
3. S. Negrini, V. G. Gorgoulis, T. D. Halazonetis, *Nat. Rev. Mol. Cell Biol.* **11**, 220 (2010).
4. C. Lengauer, K. W. Kinzler, B. Vogelstein, *Nature* **396**, 643 (1998).
5. S. Yachida *et al.*, *Nature* **467**, 1114 (2010).
6. P. J. Campbell *et al.*, *Nature* **467**, 1109 (2010).
7. Y. M. Lo *et al.*, *Sci. Transl. Med.* **2**, 61ra91 (2010).
8. J. O. Kitzman *et al.*, *Sci. Transl. Med.* **4**, 137ra76 (2012).
9. S. Nagrath *et al.*, *Nature* **450**, 1235 (2007).
10. E. K. Hanson, J. Ballantyne, *Anal. Biochem.* **346**, 246 (2005).
11. M. L. Metzker, *Nat. Rev. Genet.* **11**, 31 (2010).
12. H. C. Fan, J. Wang, A. Potanina, S. R. Quake, *Nat. Biotechnol.* **29**, 51 (2011).
13. N. Navin *et al.*, *Nature* **472**, 90 (2011).
14. M. Gundry, W. G. Li, S. B. Maqbool, J. Vijg, *Nucleic Acids Res.* **40**, 2032 (2012).
15. Y. Hou *et al.*, *Cell* **148**, 873 (2012).
16. J. Wang, H. C. Fan, B. Behr, S. R. Quake, *Cell* **150**, 402 (2012).
17. L. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5847 (1992).
18. H. Telenius *et al.*, *Genomics* **13**, 718 (1992).
19. F. B. Dean, J. R. Nelson, T. L. Giesler, R. S. Lasken, *Genome Res.* **11**, 1095 (2001).
20. K. Zhang *et al.*, *Nat. Biotechnol.* **24**, 680 (2006).
21. K. Lao, N. L. Xu, N. A. Straus, *Biotechnol. J.* **3**, 378 (2008).
22. W. Dietmaier *et al.*, *Am. J. Pathol.* **154**, 83 (1999).
23. X. Xu *et al.*, *Cell* **148**, 886 (2012).
24. R. Beroukhi *et al.*, *Nature* **463**, 899 (2010).
25. P. J. Stephens *et al.*, *Cell* **144**, 27 (2011).
26. P. J. Rochette, N. Bastien, J. Lavoie, S. L. Guérin, R. Drouin, *J. Mol. Biol.* **352**, 44 (2005).
27. D. MacArthur, *Nature* **487**, 427 (2012).
28. P. C. Blainey, S. R. Quake, *Nucleic Acids Res.* **39**, e19 (2011).
29. J. W. Drake, B. Charlesworth, D. Charlesworth, J. F. Crow, *Genetics* **148**, 1667 (1998).
30. J. C. Roach *et al.*, *Science* **328**, 636 (2010).
31. D. F. Conrad *et al.*, 1000 Genomes Project, *Nat. Genet.* **43**, 712 (2011).
32. D. L. Altshuler *et al.*, 1000 Genomes Project Consortium, *Nature* **467**, 1061 (2010).

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Supplementary Materials

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Probing Meiotic Recombination and Aneuploidy of Single Sperm Cells by Whole-Genome Sequencing

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Meiotic recombination creates genetic diversity and ensures segregation of homologous chromosomes. Previous population analyses yielded results averaged among individuals and affected by evolutionary pressures. We sequenced 99 sperm from an Asian male by using the newly developed amplification method—multiple annealing and looping-based amplification cycles—to phase the personal genome and map recombination events at high resolution, which are nonuniformly distributed across the genome in the absence of selection pressure. The paucity of recombination near transcription start sites observed in individual sperm indicates that such a phenomenon is intrinsic to the molecular mechanism of meiosis. Interestingly, a decreased crossover frequency combined with an increase of autosomal aneuploidy is observable on a global per-sperm basis.

Meiosis plays a crucial role in generating haploid gametes for sexual reproduction. In most organisms, the presence of crossovers between homologous chromosomes, in combination with connections between sister chromatids, creates a physical connection that ensures regular segregation of homologs at the first of the two meiotic divisions (1). Abnormality in generating crossovers is the leading cause of miscarriage and birth defects (2). Crossovers also create new combinations of alleles, thus contributing to genetic diversity and evolution (3).

Recent linkage disequilibrium and pedigree studies have shown that the distribution of recombination is highly uneven across the human genome (4, 5), as in all studied organisms. Substantial recombination active regions are not conserved between humans and chimpanzees (6–8) or among different human populations (9, 10), suggesting that these regions are quickly evolving and might even be individual-specific (11). However, such variation in the human population would be masked by the population average, and resolution of this variation would require comparison of recombination genome-wide among many single genomes.

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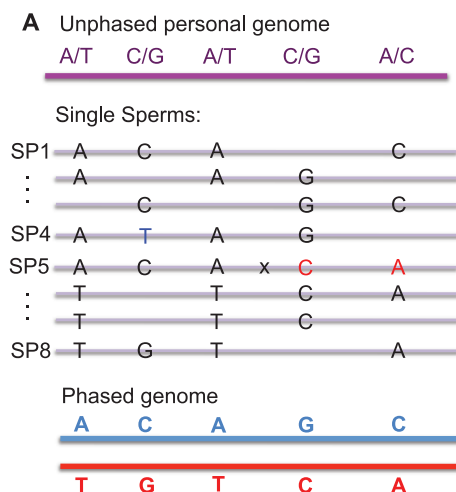
Whole-genome amplification (WGA) of single sperm cells was proposed decades ago to facilitate mapping recombination at the individual level (12). With the development of high-throughput genotyping technologies (13, 14), whole-genome mapping of recombination events in single gametes of an individual is achievable and was recently demonstrated by performing WGA by multiple displacement amplification (MDA) (15) on single sperm cells, followed by genotyping with DNA microarrays recently demonstrated by Wang *et al.* (16). However, due to the amplification bias and, consequently, insufficient marker density, the resolution of crossover locations has been limited to ~150 kb thus far. In addition, in their recent work (16),

Wang *et al.* relied on prior knowledge of the chromosome-level haplotype information of the analyzed individual, which is experimentally inconvenient to obtain and is currently available for only a few individuals (17–19).

Here, we demonstrate a more general approach for studying recombination in single sperm cells of an individual, without prior knowledge of the haplotype information. We isolated single sperm cells from a healthy Asian male donor in his late 40s. The donor has healthy offspring of both genders and normal clinical semen analysis results. We used a recently developed method, multiple annealing and looping-based amplification cycles (MALBAC) (20), to perform WGA on single cells. MALBAC provides substantially improved amplification evenness compared with the prevailing WGA methods, such as MDA. We sequenced 93 sperm at ~1× genome depth and 6 sperm at ~5× depth, achieving genome coverages of ~23 and ~43%, respectively (table S1). Three of the 99 sperm samples were found to contain more than one haploid cell and were filtered out in downstream analysis (fig. S1). Approximately 89% of the sequencing reads from single sperm can be aligned to the human genome, in agreement with that of a typical human resequencing project.

We further sequenced the diploid genome of the donor at ~70× depth and identified ~2.8 million single nucleotide polymorphisms (SNPs). About 1.4 million of them are heterozygous (hetSNPs) (table S2) (21). Among the hetSNP sites, ~500,000 (35%) and ~300,000 (20%) could be genotyped with a >99% accuracy (Phred quality score > 20) threshold for the high coverage (5×) and low coverage (1×) sperm cells, respectively (table S3).

Phase information is crucial for the correct description and interpretation of the human



B

Heterozygous SNPs	1,390,544
% covered by ≥ 2 sperm	88.0%
% phased by sperm linkage	82.4%
% consistent with family trio	99.5%
Phased length	Whole chromosome

Fig. 1. Principle of whole-genome phasing of an individual using the SNP linkage information from individual sperm cells. (A) We sequenced the diploid genome and identified five hetSNPs with

unknown linkage information shown in purple. Individual sperm cells were sequenced after MALBAC amplification, from which SNP linkage information in each sperm was used to infer the phase information in the diploid genome. (B) Performance of whole-genome phasing by SNP linkage in sperm cells.

genome (22) and is essential for mapping cross-overs. We phased the hetSNPs into chromosome-level haplotypes by comparing the SNP linkage information across all sperm (Fig. 1A) (21). Because crossovers (such as the A-C link in SP5) and false SNP identification (such as the high-lighted T in SP4) are low-probability events, most SNP linkage information identified in a sperm reflects the true SNP linkage in the somatic genome. These SNP linkages were calculated statistically by comparing across all sperm cells. In doing so, we were able to phase ~1.1 million (~82%) hetSNPs with high confidence into two sets of chromosome haplotypes. To verify the phasing result, we lightly sequenced the genomes from the donor's parents (~10× each) and used a pedigree approach to infer the phase information of the donor (tables S3 and S4) (21, 23). We obtained ~99.5% consistency between the two methods, indicating the high accuracy of our

approach in phasing hetSNPs into chromosome-level haplotypes (Fig. 1B and table S4) (21). The percentage of phased hetSNPs could be further improved with higher sequencing depths from each sperm (currently only ~1×). Several methods for haplotyping individual humans have been described previously (19, 24, 25). However, these methods often involved labor-intensive sample preparations and had limited haplotype block size (<1 Mb). Our method enables whole-genome phasing into haplotypes of complete chromosomes, without requiring cell culture or devices for separating metaphase chromosomes (18, 26). With the diploid genome phased into haplotypes of complete chromosomes, we used SNPs as markers to map the positions of crossovers in each sperm. We used a hidden Markov model to accurately determine the positions for most crossovers, and we manually identified the crossovers

for the low-confidence regions (Fig. 2A) (21). We identified 2368 autosomal crossover events in the sperm cells with a complete haploid genome. The average of ~26.0 crossovers per cell is consistent with reported pedigree studies (27, 28). The amplification evenness of MALBAC allowed us to achieve high resolution in detecting crossovers with only ~1× sequencing depth from each sperm. About 93, 80, and 45% of the crossovers can be confidently assigned to intervals of 200, 100, and 30 kb, respectively (Fig. 2B), compared with 59, 37, and 13% from the recently reported single-sperm study (16). Of the crossovers unambiguously resolvable within a 10-kb interval, ~40% are found to overlap with the male-specific recombination hotspots inferred from the deCODE project (9). Also, ~45% of these crossovers are close to the PRDM9 binding motif CCnCCnTnnCCnC, which is consistent with previous population studies (29).

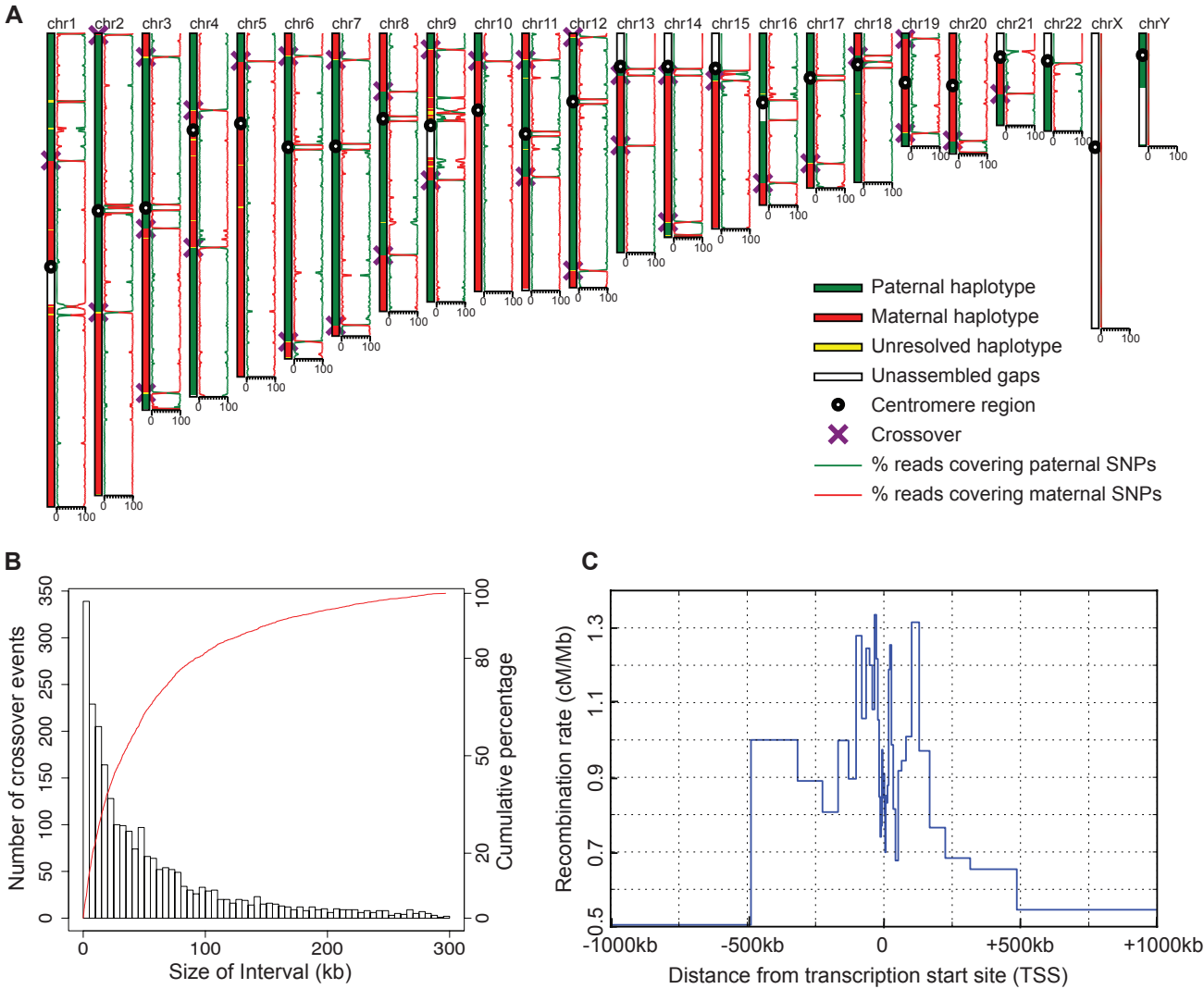


Fig. 2. Identifying crossover positions in individual sperm cells. **(A)** Parental haplotype contributions are determined by comparing the percentage of reads covering the paternal or maternal SNPs, and crossover positions are detected by identifying the crossing locations of the two

parental haplotypes by a hidden Markov model. **(B)** Resolution of crossover determination. About 60% of the crossovers can be determined within intervals of 50 kb. **(C)** Distribution of recombination rate relative to TSSs. cM, centimorgans.

Recombination rates correlate positively with gene density in both yeast and humans (27, 30). However, at a finer scale, recombination rates in human populations are lower very close to genes (within 20 kb) and higher tens or hundreds of kilobases away from the transcription start sites (TSSs) (4, 9, 28). This feature is an average of different individuals that reflects the cumulative effect of human evolutionary history, and it may also be complicated by selecting against the recombinations that compromise offspring viability. Our method detects recombination features based on single gametes, which are free of selection effects of population studies. We analyzed the crossovers resolvable within 30 kb and derived the recombination rate relative to the TSSs of the individual (Fig. 2C) (21). We observed lower recombin-

ation rates close to the TSSs and higher rates tens of kilobases away, consistent with the results of previous population studies (4, 9, 28), indicating that the reduced recombination rate close to TSSs is primarily due to the variation of recombination probability during meiosis rather than due to selection.

Previous population studies have shown that recombination events have a nonuniform distribution across the genome, which reflects the cumulative evolutionary history of recombination (5). By binning the crossover incidence into 3-Mb units in autosomes, we constructed a genetic map of recombination of the individual. We compared our map to a sex-averaged map based on population (HapMap) (4) and a male-specific map based on pedigree (deCODE) (9) (Fig. 3, A and B) and obtained correlation coefficients

of 0.71 and 0.77, respectively. In some of the bins, we observed a significant difference between HapMap and the donor (table S6), which can be explained by sex-specific recombination variations.

A recent study reported that crossover active regions that are specific to an individual exist at a megabase scale (16). We also found nine bins showing significant differences between the donor and deCODE (table S7). However, we note that most of these regions are very close to the centromere or the ends of the chromosomes, where the estimation of the recombination rates was considered unreliable and excluded in deCODE (9). Therefore, we suspect that these differences mainly reflect the incompleteness of the deCODE database. Our results suggest that the distribution of recombination in

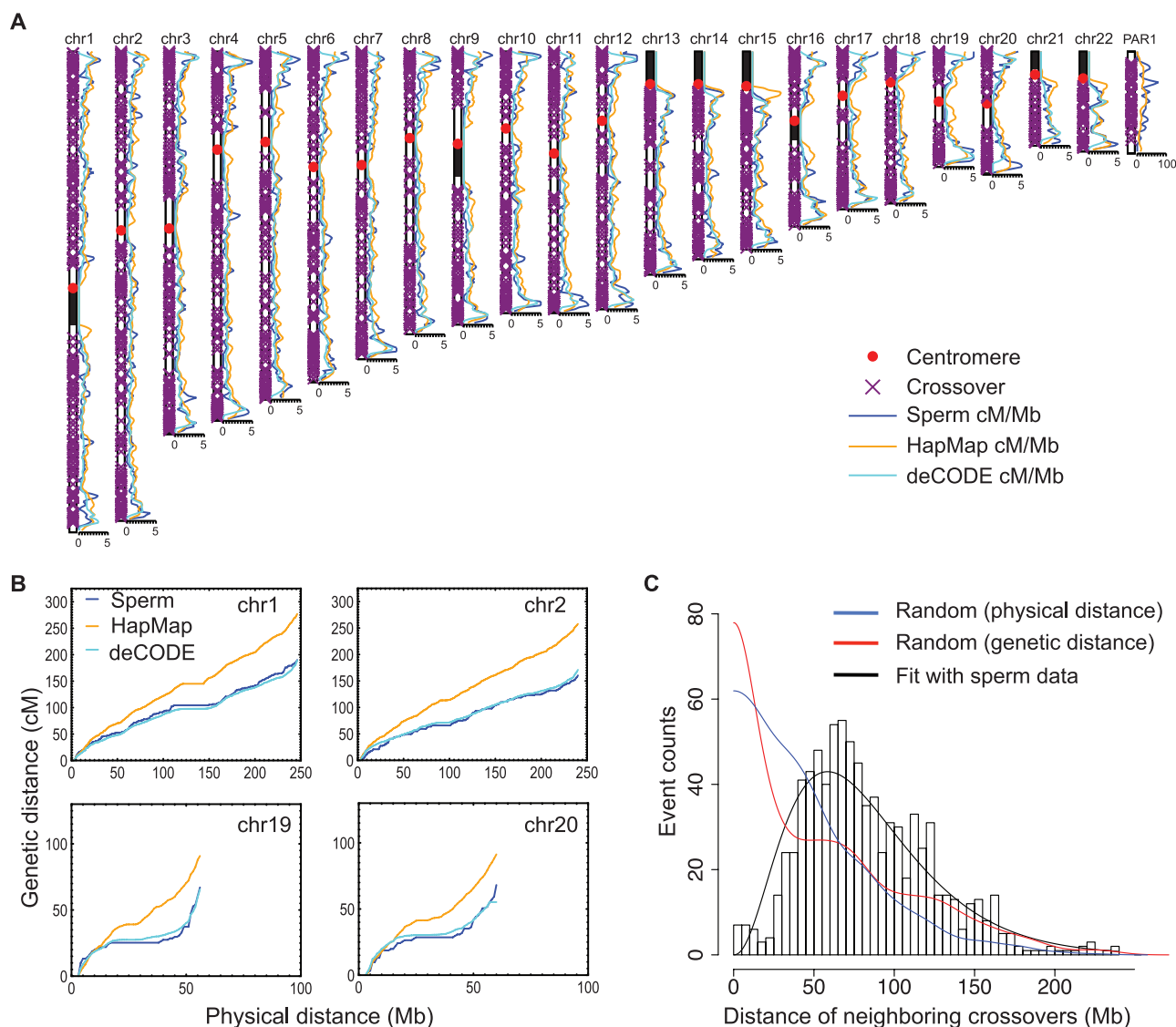
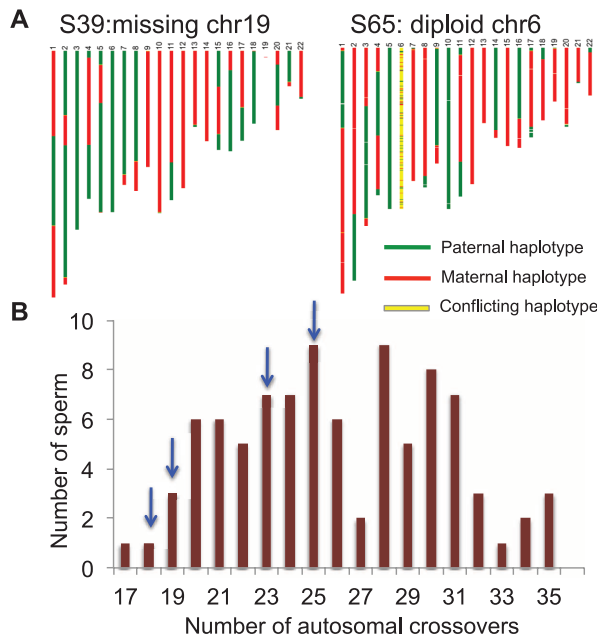


Fig. 3. Genome-wide distribution of recombination. **(A)** Comparison of the sperm recombination rates to the HapMap and deCODE (male-specific) genetic maps across the human genome. We used a 3-Mb statistical window size and a 1-Mb moving step. **(B)** A personal genetic map. Relations of physical and genetic length of selected chromosomes. **(C)**

Distance distribution of crossovers co-occurring on the same chromosome. The experimental data are fit with a gamma distribution ($\alpha = 3.35$), indicating a strong deviation from the random distribution. In comparison, we generated random crossovers based on physical and genetic distances.

Fig. 4. Detecting aneuploidy and crossover in the same sperm. **(A)** Two of the four sperm cells that exhibit autosomal abnormality. Few reads are mapped to chr19 in S39, indicating a loss of chr19. Both parental haplotypes are found in chr6 of S65, indicating a disomy chr6 in the sperm. The detailed coverage analysis on all four aneuploid sperm is shown in fig. S2. **(B)** Distribution of the autosomal crossover number. Arrows indicate the number of crossovers in sperm cells with autosomal aneuploidy.



the individual generally agrees with the population average at the megabase scale, indicating a general consistency of large-scale recombination distribution in human evolution. With the rapid development of sequencing technologies, more sperm can be analyzed in the future from different individuals to look into fine-scale recombination differences. We estimated that at least 1000 sperm are required to identify personal recombination differences with statistical significance (fig. S4) (21).

Obtaining the genome sequence of each sperm also allowed us to examine the coexisting crossovers on the same chromosome. The adjacent crossovers exhibit longer distances than expected by random chance (Fig. 3C and figs. S5 and S6), which is consistent with the well-established phenomenon of crossover interference (31, 32). Although we have higher resolution to detect crossovers than in a previous study, we did not see the reported phenomenon of substantial double crossovers occurring close together (e.g., 1 to 5 Mb) (33), which suggests that such phenomenon is likely not general and may only exist in certain populations.

Failure to form crossovers during meiosis gives rise to chromosome segregation errors that result in aneuploidy. Autosomal aneuploidy is often lethal to embryos, with the exception of a few chromosomes that result in severe health consequences early in development (e.g., trisomy 21, Down syndrome). Reduced recombination activity is often associated with male infertility and sperm aneuploidy (34). By comparing the coverage depth and SNPs along the genome of the sperm cells, we detected four cells either missing or having additional autosomes (Fig. 4A and fig. S2) (21). The rate of chromosome mis-segregation is consistent with the reported imaging studies on selected loci of human spermatocytes (35, 36).

We next compared the crossover number of the aneuploid sperm to the normal sperm. Interestingly, sperm cells with aneuploid autosomes exhibit significantly fewer crossovers than normal cells, on average ($P = 0.01$). Our result suggests that autosomal segregation errors are not generated randomly during spermatogenesis. Instead, the error rate is higher in the spermatocytes with relatively repressed crossover activity. However, such a trend does not seem to be significant for sex chromosome aneuploidy, as we observed a sperm with 30 autosomal crossovers but no sex chromosome. Indeed, the crossover probability in the pseudo-autosomal region of the sex chromosomes has no noticeable correlation with that of the autosomes (tables S8 and S9) (21), suggesting a different mechanism of crossover generation for autosomes and sex chromosomes, which is consistent with an earlier study in mice (37). We were unable to determine whether the chromosomes exhibiting aneuploidy underwent recombination, as recombination events are observable only when a single copy is present. MALBAC allows direct examination of meiotic crossovers and chromosome segregation errors on a per-meiotic nucleus basis at high resolution, enabling further applications for the study of genome instability and male infertility.

References and Notes

1. M. Petronczki, M. F. Siomos, K. Nasmyth, *Cell* **112**, 423 (2003).
2. C. J. Epstein, *The Consequences of Chromosome Imbalance: Principles, Mechanisms, and Models* (Cambridge Univ. Press, Cambridge, 2007).
3. A. J. Jeffreys, C. A. May, *Nat. Genet.* **36**, 151 (2004).
4. S. Myers, L. Bottolo, C. Freeman, G. McVean, P. Donnelly, *Science* **310**, 321 (2005).
5. K. Paigen, P. Petkov, *Nat. Rev. Genet.* **11**, 221 (2010).

6. W. Winckler *et al.*, *Science* **308**, 107 (2005).
7. S. E. Ptak *et al.*, *Nat. Genet.* **37**, 429 (2005).
8. A. Auton *et al.*, *Science* **336**, 193 (2012).
9. A. Kong *et al.*, *Nature* **467**, 1099 (2010).
10. A. G. Hinch *et al.*, *Nature* **476**, 170 (2011).
11. A. J. Jeffreys, R. Neumann, *Nat. Genet.* **31**, 267 (2002).
12. L. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5847 (1992).
13. D. J. Lockhart, E. A. Winzler, *Nature* **405**, 827 (2000).
14. M. L. Metzker, *Nat. Rev. Genet.* **11**, 31 (2010).
15. F. B. Dean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 5261 (2002).
16. J. Wang, H. C. Fan, B. Behr, S. R. Quake, *Cell* **150**, 402 (2012).
17. S. Levy *et al.*, *PLoS Biol.* **5**, e254 (2007).
18. H. C. Fan, J. Wang, A. Potanina, S. R. Quake, *Nat. Biotechnol.* **29**, 51 (2011).
19. B. A. Peters *et al.*, *Nature* **487**, 190 (2012).
20. C. Zong, S. Lu, A. R. Chapman, X. S. Xie, *Science*, **338**, 1622 (2012).
21. Materials and methods, as well as other supplementary materials, are available on Science Online.
22. R. Tewhey, V. Bansal, A. Torkamani, E. J. Topol, N. J. Schork, *Nat. Rev. Genet.* **12**, 215 (2011).
23. S. R. Browning, B. L. Browning, *Nat. Rev. Genet.* **12**, 703 (2011).
24. J. O. Kitzman *et al.*, *Nat. Biotechnol.* **29**, 59 (2011).
25. E.-K. Suk *et al.*, *Genome Res.* **21**, 1672 (2011).
26. L. Ma *et al.*, *Nat. Methods* **7**, 299 (2010).
27. A. Kong *et al.*, *Nat. Genet.* **31**, 241 (2002).
28. G. Coop, X. Wen, C. Ober, J. K. Pritchard, M. Przeworski, *Science* **319**, 1395 (2008).
29. S. Myers, C. Freeman, A. Auton, P. Donnelly, G. McVean, *Nat. Genet.* **40**, 1124 (2008).
30. T. D. Petes, *Nat. Rev. Genet.* **2**, 360 (2001).
31. N. Kleckner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12592 (2004).
32. M. A. Handel, J. C. Schimenti, *Nat. Rev. Genet.* **11**, 124 (2010).
33. A. Fedel-Alon *et al.*, *PLoS Genet.* **5**, e1000658 (2009).
34. K. A. Ferguson, E. C. Wong, V. Chow, M. Nigro, S. Ma, *Hum. Mol. Genet.* **16**, 2870 (2007).
35. E. L. Spriggs, A. W. Rademaker, R. H. Martin, *Cytogenet. Cell Genet.* **71**, 47 (1995).
36. S. E. Downie, S. P. Flaherty, N. J. Swann, C. D. Matthews, *Mol. Hum. Reprod.* **3**, 815 (1997).
37. L. Kauppi *et al.*, *Science* **331**, 916 (2011).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6114/1627/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S9
References

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Organization of the Influenza Virus Replication Machinery

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Influenza virus ribonucleoprotein complexes (RNPs) are central to the viral life cycle and in adaptation to new host species. RNPs are composed of the viral genome, viral polymerase, and many copies of the viral nucleoprotein. In vitro cell expression of all RNP protein components with four of the eight influenza virus gene segments enabled structural determination of native influenza virus RNPs by means of cryogenic electron microscopy (cryo-EM). The cryo-EM structure reveals the architecture and organization of the native RNP, defining the attributes of its largely helical structure and how polymerase interacts with nucleoprotein and the viral genome. Observations of branched-RNP structures in negative-stain electron microscopy and their putative identification as replication intermediates suggest a mechanism for viral replication by a second polymerase on the RNP template.

Influenza A virus is a single-stranded RNA virus that causes frequent epidemics as well as sporadic pandemics (*1*). The constant threat of pandemic influenza is highlighted by the emergence of novel pandemic H1N1 viruses in 2009 (*2*) and the potential for highly pathogenic avian H5N1 viruses to gain human-to-human transmissibility (*3*). Several studies on influenza virus have pointed to components of the ribonucleoprotein complex (RNP) as key factors in host adaptation (*4*). The RNPs are responsible for viral transcription and replication as well as assembly of the genome segments into progeny virions (*1*).

The RNP comprises a single polymerase bound to the complementary RNA termini and multiple copies of the viral nucleoprotein (NP) that decorate the length of each of the eight single-stranded viral genome segments, so that the RNP resembles a large loop, twisted into a helical filament (*5*). The RNA polymerase is composed of PB1, the catalytic subunit, and PB2 and PA subunits, which carry activities for priming transcription (*1, 6, 7*). Some fragment crystal structures have been determined (*8*), but how the subunits form a functional polymerase, interact with NP and the viral genome, or modulate interactions with host factors is still unclear.

Oligomerization of NP into a nonphysiologic trimer, as observed in crystal structures, is facilitated by insertion of a long “tail loop” from each NP monomer into a binding site on the adjacent monomer (*9*). The tail loop has been shown biochemically to be important for oligomerization of NP monomers within the RNP (*10–12*), but structural information on the native RNP has been

lacking, and it is unclear how the NPs form the helical filament structures that are characteristic of influenza virus RNPs (*5*). Electron microscopy (EM) studies have previously been performed on constrained mini-RNPs (*10*) via truncation of the RNA genome to 254 nucleotides (nt). The mini-RNP is not expected to recapitulate all of the interactions of native RNP complexes, which contain 890 to 2341 nt, particularly in formation of native-like filaments. To address the plethora of biological questions surrounding the influenza virus RNP, we used cryogenic electron microscopy (cryo-EM) to analyze the structure of recombinant RNPs.

The RNPs were derived from influenza virus gene segments 1, 2, 3, and 5, which encode the protein components of the RNP complex, using a plasmid-based system (*13*). The coexpression of RNP protein components with viral genomes facilitates in vivo assembly of native RNP complexes with viral transcription and replication activity. The RNPs in the electron micrographs are highly flexible along their length (fig. S1) but display a regular diameter and repeat allowing three-dimensional (3D) reconstruction of the three main regions: the RNA-polymerase end, the central filament, and the looped end (Fig. 1, A and B, and table S1) (*5, 14*).

Reconstruction of the central filament region was performed by selecting short, overlapping regions along the length of the RNP and applying helical symmetry based on initial analysis of non-symmetrized reconstructions. To reconstruct both ends of the RNP, putative RNP end regions (blunt and loop ends) were identified, selected, and subsequently classified using a 3D maximum likelihood approach (*15, 16*) to separate images of the loop end from the polymerase end. A polymerase in isolation reconstruction was obtained by digesting and disassembling the RNPs with ribonuclease. The 3D maps are low-pass filtered at a resolution indicated by the Fourier shell coefficient curve at 0.5. Back projections of all 3D reconstructions are in good agreement with their corresponding 2D class averages (figs. S2 to S5).

The 3D cryo-EM reconstruction of a 23-nm segment of the central region of the RNP filament at 21 Å resolution (Fig. 1C) indicates that the NP-RNA complex forms two antiparallel strands that twist about one another to and from the RNA polymerase, which is bound to the RNA termini (*5*). Single-NP protomers derived from the crystal structure (*9*) were fit into the EM map with the aid of automated docking procedures by using structural, biological, and symmetry constraints (Fig. 1D and movie S1) (*17*). Influenza virus is unusual among RNA viruses in that transcription and replication occur within the cell nucleus (*1*). In the fitted model, the NP N-terminal nuclear localization signal (NLS I) is exposed, whereas a second putative NLS (NLS II) is buried in the protein-protein interface (fig. S6), which is in agreement with antibody-labeling studies (*18*). Although the modest resolution map does not allow an atomic interpretation, the protomer arrangement and regions of the NP can be localized within the RNP. The model is consistent with the finding (*19*) that the NP tail loop is used for oligomerization along the NP-RNA strand (fig. S6) and the loss of transcriptional activity when residues in the NP tail-loop are mutated

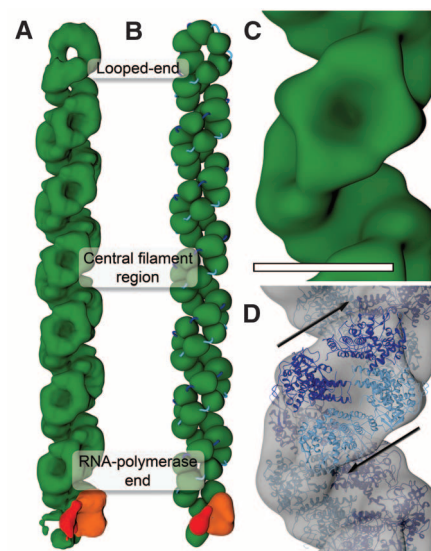


Fig. 1. Cryo-EM reconstruction of the influenza virus RNP. (A) Composite model of cryo-EM reconstructions of the three regions of the RNP. (B) Illustration of the RNP organization. The large domain of polymerase is shown in orange, with the arm domain in red. NPs are shown in green, and RNA is shown in blue. (C) Reconstruction of the central filament region using helical symmetry. (D) Single protomers from the NP crystal structure (*9*) were fitted into the EM density. The arrangement of the NP (light blue for descending strand and dark blue for ascending strand) within the filament creates a periodic box-type arrangement formed from four NPs, with a region of low density or dimple in the center of the box. This box-like feature is also easily identifiable in our reconstructions of the loop and polymerase end regions. Arrows indicate RNA polarity. Scale bar, 10 nm.

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(fig. S7) (10–12). The RNP helix is stabilized by one NP-RNA strand interacting with the antiparallel NP-RNA strand near the NP head domains (fig. S8) (20). The NP orientation also directs the proposed NP RNA-binding regions into the inter-strand NP-NP interface so that the RNA sequences that are most intimately bound by NP are not directly accessible for transcription or replication and suggests that at least local disassembly of the RNP is required for these processes to occur.

In this central region, adjacent NP protomers rise by 32.6 Å and twist by 73.9° for an average of 4.9 NP along the RNA strand in one turn of the helix (21). The average periodicity of NP on the genomic RNA is 32 ribonucleotides (22), which is similar to the 26- to 32-nt periodicity range calculated previously from constrained mini-RNP (10, 23, 24). The average NP-binding periodicity of the native RNP is understandably somewhat larger because of its more relaxed conformation. Furthermore, the spacing of the NP RNA-binding sites leaves large sections of RNA exposed (fig. S9), which explains the susceptibility of influenza virus RNP to ribonucleases (5). Some of the exposed RNA may present terminal genome assembly signals to facilitate interactions with the other gene segments and the assembly of the viral genome into progeny virions (25). These signals would be presented within about six NPs, or 19 nm, from the polymerase end of the RNP, which is in agreement with recent influenza virus tomographic studies in which interactions between the assembled eight RNPs are observed within 13 to 17 nm of the RNP filament end (26, 27).

The loop end of the RNP was reconstructed from cryo-EM images to a resolution of ap-

proximately 40 Å and demonstrates that the NP-RNA strand makes a sharp U-turn to and from the central filament region (Fig. 1A and fig. S10). 2D averages of negatively stained RNP show the loop comprises 5 to 8 NP monomers. The observed variation is likely due to different degrees of unwinding of the central filament region.

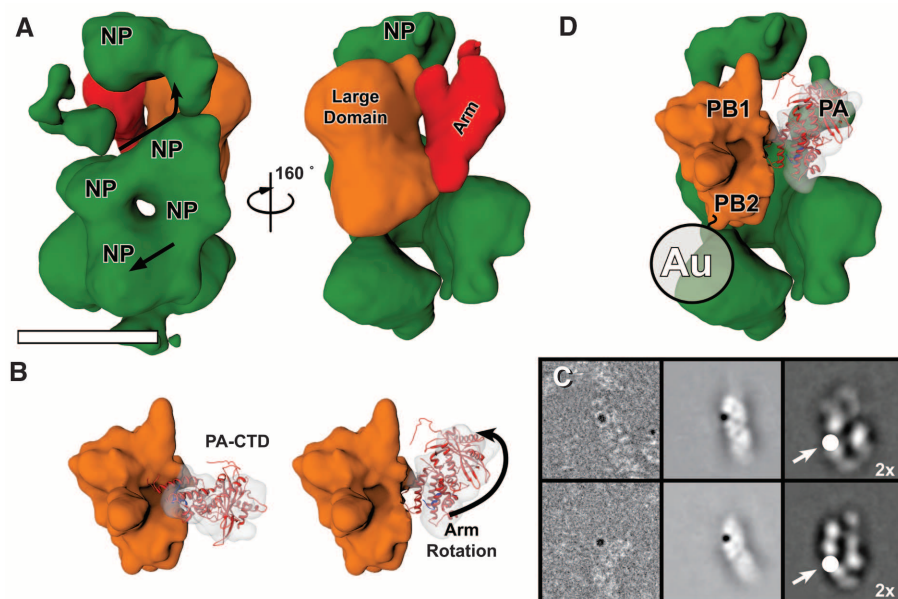
The polymerase-bound blunt end of the RNP was reconstructed to a resolution of 20 Å from the cryo-EM images. The heterotrimeric RNA-dependent RNA polymerase has two primary domains: a large domain and a smaller arm domain. Polymerase directly contacts two NPs on its large domain (Fig. 2A and fig. S10) and is adjacent to an additional NP monomer. Our reconstruction is similar to previously published densities (10); however, we identify a mass previously assigned to polymerase as more likely representing the adjacent additional NP monomer (fig. S11). The structure of purified polymerase was determined with cryo-EM to 13 Å resolution (28). This higher-resolution structure could be fitted into the native RNP-bound polymerase reconstruction after rearrangement of the arm domain (Fig. 2B). Conformational isomerism of the arm domain is consistent with observations made for polymerases of other segmented negative-sense RNA viruses (fig. S12) (29). The arm domain is of a size and shape that suggests it is the PA C-terminal domain (PA-CTD) (30).

To locate the PB2 polymerase subunit within the RNP complex, the C-terminus of the PB2 polymerase subunit in RNPs was labeled with 5-nm Nanogold (Nanoprobes, Yaphank, NY) and negatively stained. The gold label localizes to the base of the polymerase large domain near the

contact site of the NP antiparallel helical screw (Fig. 2C). The PB2 “627” domain is located adjacent to the PB2 C-terminus (31). Avian viruses lacking adaptive mutations on the surface of this domain, particularly Glu⁶²⁷Lys, are inhibited by a mammalian host factor that interferes with association of the avian polymerase with NP (32–34). Our identification of the 627 domain near the NP-binding site on polymerase suggests a direct competition for polymerase binding between NP and the mammalian inhibitory host factor, leading to host restriction for most avian viruses.

The NP protomers can be used as markers of the viral RNA genome path near the polymerase because the viral RNA is not observable at the resolution of our reconstruction. One NP-RNA strand contacts polymerase near the PB2 C-terminus, whereas the second NP-RNA strand departs from the helical filament to loop around and then contact polymerase via the PA-CTD (fig. S10). This organization places the second RNA strand on the putative RNA-binding site of the PA-CTD (30). Arg⁵⁶⁶Glu or Lys⁵⁷⁴Glu mutations in this binding site completely abolish transcriptional activity (fig. S7). Structural homology of the PA-CTD with the N-terminal domain of reovirus RNA polymerase (30, 35) suggests that this second strand is the 3' end of the genome, which is directed toward the polymerase active site on the polymerase large domain; therefore, the first strand is the 5' end (fig. S13). The partially base-paired RNA termini must be proximal to the polymerase active site to place a 5' proximal polyuridine stretch across the active site while remaining bound to the 5' terminus and facilitate the proposed “stuttering” mechanism of polyadenylation (Fig. 3A) (36).

Fig. 2. The influenza virus RNA polymerase and its interactions at the RNP terminus. **(A)** The RNP-bound polymerase large domain (orange) caps the central RNP filament region. A single NP from the second strand that has emerged from the central filament follows the 3' RNA strand as it loops behind polymerase to contact the PA-CTD (red). **(B)** Cryo-EM reconstruction of the free polymerase (left) shows it consists of a large domain (orange) and an arm containing the PA-CTD (red) (30). The arm domain conformation in the RNP is accommodated by a rotation about a pivot near the base of the arm (right). **(C)** 2D averages and raw images of negatively stained RNPs labeled with 5-nm Nanogold (left and middle, respectively) localize the PB2 C-terminus to the bottom of the large domain near the NP contact site. (Right) 2D projections of 21 Å RNP-bound polymerase reconstruction with an additional circle (indicated with an arrow) below the polymerase-large domain corresponding to the size of the 5-nm Nanogold for comparison with labeled 2D class averages. **(D)** The composite image of the RNP polymerase end has been labeled to indicate putative subunit locations based on the PA-CTD docking, Nanogold labeling of the PB2 C-terminus, and structural homology with reovirus polymerase. The location of the 5-nm Nanogold labeling the PB2 C-terminus is shown as a circle labeled Au.



The placement of the complementary RNA termini on the upper portion of the polymerase large domain is supported by a previous reconstruction of the polymerase bound to small RNA (37). These data support a model of RNA synthesis in which PA-CTD plays a role in feeding template RNA into the polymerase active site.

On the basis of our structural models and incorporating additional structural and biochemical results from the literature, we can propose a model for viral transcription from influenza virus RNPs (Fig. 3B, fig. S14, and movie S2). In the preinitiation state, the RNA termini are base-paired adjacent to the polymerase active site. The PA and PB2 subunits bind and cleave host mRNA to produce capped primers for transcription initiation. The 3' end then disengages from the 5' end and repositions to the polymerase active site. As transcription proceeds, the PA-CTD moves single-stranded RNA from the RNP filament into the active site. As transcription reaches the 5' end of the template and the RNA noose tightens, the 5' terminus remains bound to the polymerase, positioning the polyuridine stretch across the active site. The polymerase stutters across this region to bring about polyadenylation and transcription termination.

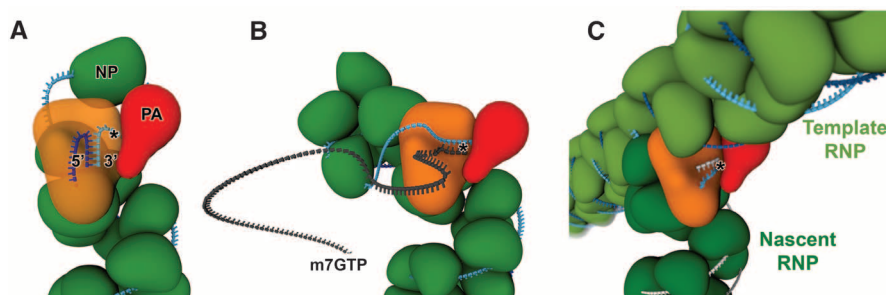
Replication of the influenza virus genome is distinct from transcription in that the RNA product is not capped or polyadenylated. Viral

replication involves the production of an intermediate complementary RNP (cRNP) complex composed of complementary genomic RNA (cRNA), polymerase, and NP. The cRNP then acts as a template for viral genome synthesis. During replication, the RNP is thought to act only as a template, with RNA polymerization carried out by a second polymerase (38). Examination of negative-stain EM micrographs reveals numerous RNP complexes with a branched arrangement, in which a smaller nascent RNP appears to bud from a larger full-length RNP (Fig. 4). Nascent RNPs with very small lengths, half length, or near full length are most common near the RNP filament ends, which is as expected for a nascent RNP replicating on a full-length RNP template (fig. S15). Additionally, in RNP samples containing labeled polymerase, we observe polymerase, residing at the junction of the smaller RNP with the full-length RNP (fig. S16). The observation of branched RNPs and their putative identification as replication intermediates is consistent with previous biochemical and structural investigations of vesicular stomatitis virus RNPs isolated from infected cells (39). These data support a model of influenza virus replication in which a second polymerase acts on a template RNP leading to the formation of nascent RNP complexes concurrent with viral replication. It remains unclear how the second polymerase

initiates replication. However, in our model, as replication proceeds a new 5' end is synthesized and is bound by the second replicating polymerase (Fig. 3C, fig. S17, and movie S3). After 5' terminus binding, the first NP protomer is added to the product RNA adjacent to the replicating polymerase's PB2 627 domain, initiating encapsidation of the viral genome or cRNA in a 5' to 3' direction and forming a nascent RNP on the template RNP, giving the complex its branched appearance (Fig. 3C). This model describes NP encapsidation of the viral genome being initiated by sequence-specific binding of polymerase to the RNA 5' end (40), concurrent with viral replication, which would account for NP encapsidation of viral RNA and not host RNA during viral infection.

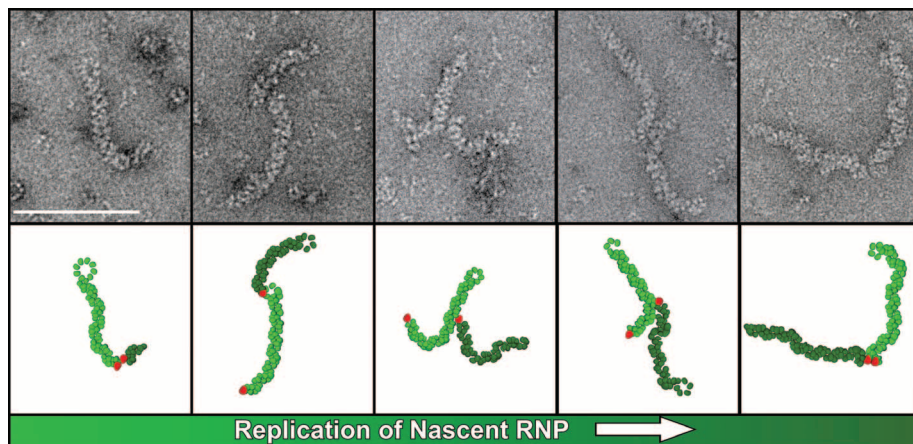
These studies provide new insights into influenza virus transcription, replication, and host species adaptation and shed light on RNP nuclear translocation and virus assembly. These findings also suggest that the PB2-NP interaction and the PA-CTD conformational rearrangement are potential targets for novel therapeutics and demonstrate the utility of targeting the NP tail loop binding site (12). Our model also provides a framework to develop new hypotheses to address how viral proteins and host factors interact with RNP for nuclear export and localization to the plasma membrane for virus assembly.

Fig. 3. Models for influenza virus RNA synthesis. **(A)** In the resting RNP, polymerase is bound to both 5' and 3' termini, as would be expected in virions. The polymerase large domain and arm domain are colored orange and red, respectively. The nucleoprotein is in green, and the genomic RNA is in light blue for the 3' end and dark blue for the 5' end. The active site (asterisk) and RNA polarity were identified by using structural homology with the reovirus $\lambda 3$ polymerase (fig. S13). **(B)** Viral transcription of mRNA is carried out by the resident polymerase. In this process, template RNA is pulled up from beneath polymerase; passed through the active site, where it is transcribed into capped mRNA (black); and then reencapsidated into a NP-RNA complex, which coils up to form an RNP-like structure. **(C)** Replication of viral RNA is carried out by a second polymerase,



leading to nascent RNP formation. The second replicating polymerase binds a newly synthesized 5' end and initiates encapsidation of the viral genome by NP in a 5' to 3' manner, leading to nascent RNP formation.

Fig. 4. Formation of nascent RNP during replication. **(Top)** Electron micrographs of the negatively stained RNP sample at high dilution reveal branched RNPs predicted to be replication intermediates. **(Bottom)** Interpretation of the branched RNPs in the top panel showing how replication of the viral RNA by a second polymerase (red) could result in nascent RNP complexes (dark green) branching from the template RNP (light green). As the nascent RNP moves away from and then back toward the template RNP polymerase—that is, 3' to 5' along the genomic template—it would elongate the cRNA and extend its length. Scale bar, 100 nm.



References and Notes

1. B. N. Fields, D. M. Knipe, P. M. Howley, *Fields' Virology* (Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, 2007).
2. G. Neumann, T. Noda, Y. Kawaoka, *Nature* **459**, 931 (2009).
3. M. Hatta, P. Gao, P. Halfmann, Y. Kawaoka, *Science* **293**, 1840 (2001).
4. G. Gabriel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18590 (2005).
5. R. W. Compans, J. Content, P. H. Duesberg, *J. Virol.* **10**, 795 (1972).
6. D. Guilligay *et al.*, *Nat. Struct. Mol. Biol.* **15**, 500 (2008).
7. P. Yuan *et al.*, *Nature* **458**, 909 (2009).
8. R. W. Ruigrok, T. Cr  pin, D. J. Hart, S. Cusack, *Curr. Opin. Struct. Biol.* **20**, 104 (2010).
9. A. K. Ng *et al.*, *FASEB J.* **22**, 3638 (2008).
10. R. Coloma *et al.*, *PLoS Pathog.* **5**, e1000491 (2009).
11. Z. Li *et al.*, *J. Virol.* **83**, 4153 (2009).
12. Y. F. Shen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 16515 (2011).
13. E. Hoffmann, G. Neumann, Y. Kawaoka, G. Hobom, R. G. Webster, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6108 (2000).
14. Materials and methods for particle expression and image analysis are available as supplementary materials on Science Online
15. C. O. Sorzano *et al.*, *J. Struct. Biol.* **148**, 194 (2004).
16. S. H. Scheres *et al.*, *J. Mol. Biol.* **348**, 139 (2005).
17. E. F. Pettersen *et al.*, *J. Comput. Chem.* **25**, 1605 (2004).
18. W. W. Wu, L. L. Weaver, N. Pant  , *J. Mol. Biol.* **374**, 910 (2007).
19. Q. Ye, R. M. Krug, Y. J. Tao, *Nature* **444**, 1078 (2006).
20. Mutations in the head domain interfaces did not show defects in transcriptional activity (fig. S7). However, mutation of the helical strand interface results in defective RNP assembly (fig. S8). These results are consistent with previous work in which NP mutants in this region retained wild-type transcription activity in mini-genome assays but could not be rescued as a virus (11).
21. Given the slight variations in RNP helical rise and twist, reported helical parameters likely represent average values.
22. The 32-nt NP periodicity was calculated from an RNA segment length of approximately 2300 nt and a total of 69 NP within the RNP, which was estimated from the end region reconstructions, the helical rise (3.26 nm), and total RNP length (110 nm).
23. J. Ortega *et al.*, *J. Virol.* **74**, 156 (2000).
24. The 26- to 32-nt periodicity of NP binding is calculated from (23) and is based on mini-RNPs having a 350-nt genome bound to 10 to 12 NP.
25. E. C. Hutchinson, J. C. von Kirchbach, J. R. Gog, P. Digard, *J. Gen. Virol.* **91**, 313 (2010).
26. E. Fournier *et al.*, *Nucleic Acids Res.* **40**, 2197 (2012).
27. T. Noda *et al.*, *Nat. Commun.* **3**, 639 (2012).
28. The density is similar to that published earlier at lower resolution (41). The previously noted large cleft in the polymerase resolves into a space between the polymerase large and arm domains (fig. S12).
29. P. J. Kranzusch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 20069 (2010).
30. X. He *et al.*, *Nature* **454**, 1123 (2008).
31. F. Tarendeau *et al.*, *PLoS Pathog.* **4**, e1000136 (2008).
32. K. Labadie, E. Dos Santos Afonso, M. A. Rameix-Welti, S. van der Werf, N. Naffakh, *Virology* **362**, 271 (2007).
33. A. Mehle, J. A. Doudna, *Cell Host Microbe* **4**, 111 (2008).
34. M. A. Rameix-Welti, A. Tomoiu, E. Dos Santos Afonso, S. van der Werf, N. Naffakh, *J. Virol.* **83**, 1320 (2009).
35. Y. Tao, D. L. Farsetta, M. L. Nibert, S. C. Harrison, *Cell* **111**, 733 (2002).
36. G. X. Luo, W. Luytjes, M. Enami, P. Palese, *J. Virol.* **65**, 2861 (1991).
37. P. Resa-Infante, M. A. Recuero-Checa, N. Zamarre  o, O. Llorca, J. Ort  n, *J. Virol.* **84**, 10477 (2010).
38. N. Jorba, R. Coloma, J. Ort  n, *PLoS Pathog.* **5**, e1000462 (2009).
39. C. W. Naeve, D. F. Summers, *J. Virol.* **34**, 764 (1980).
40. F. Baudin, C. Bach, S. Cusack, R. W. Ruigrok, *EMBO J.* **13**, 3158 (1994).
41. E. Torreira *et al.*, *Nucleic Acids Res.* **35**, 3774 (2007).

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Supplementary Materials

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The Structure of Native Influenza Virion Ribonucleoproteins

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The influenza viruses cause annual epidemics of respiratory disease and occasional pandemics, which constitute a major public-health issue. The segmented negative-stranded RNAs are associated with the polymerase complex and nucleoprotein (NP), forming ribonucleoproteins (RNPs), which are responsible for virus transcription and replication. We describe the structure of native RNPs derived from virions. They show a double-helical conformation in which two NP strands of opposite polarity are associated with each other along the helix. Both strands are connected by a short loop at one end of the particle and interact with the polymerase complex at the other end. This structure will be relevant for unraveling the mechanisms of nuclear import of parental virus RNPs, their transcription and replication, and the encapsidation of progeny RNPs into virions.

The influenza A viruses belong to the *Orthomyxoviridae* family and cause annual epidemics and occasional pandemics of respiratory disease. The latest pandemic was originated by low-pathogenic H1N1 viruses (1), but fears remain that high-pathogenic H5N1 avian viruses may cause a catastrophic pandemic. The viral genome contains eight single-stranded negative-polarity RNAs assembled into ribo-

nucleoprotein particles (RNPs) containing the viral polymerase and multiple nucleoprotein (NP) monomers (2, 3); within each RNP, the RNA adopts a closed conformation by interaction of both ends with the polymerase. Viral RNPs act as independent molecular machines for transcription and replication in the nucleus, both processes being mediated by the heterotrimeric polymerase complex. Viral mRNAs are produced by cap-

snatching, whereas RNA replication generates progeny RNPs (2–4), which are encapsidated in an ordered manner into virus particles (5, 6). The RNPs are flexible, helical particles (7, 8) with general features determined by the NP (9), which contain the polymerase at one end (10, 11) and a closing loop at the other. The atomic structure of isolated RNP components, including NP and several polymerase subunit domains, has been solved recently (3, 4, 12, 13), but its detailed quaternary organization to form virion RNPs remains unknown.

Previous studies using recombinant RNPs identified circular, elliptical, or helical structures depending on the RNA length (14). Circular RNPs were studied by cryogenic electron microscopy (cryo-EM), providing the first description of biologically relevant NP-NP and NP-polymerase interactions (15, 16). However, these particles lacked

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the helical features typical of virion RNPs that might be important for proper replication, transcription, and packaging. Unfortunately, the length heterogeneity and high flexibility of virion RNPs have hampered any structural analysis so far. To overcome these problems, we performed separate analyses of termini and central regions of RNPs isolated from purified A/WSN virions (Fig. 1A) by either negative staining or cryo-EM. Images from central regions or RNP termini were separately classified by using a pattern-free algorithm, and representative two-dimensional (2D) averages are presented in Fig. 1, B to D, and fig. S1. The class-average images from central regions were compatible with a helical structure (Fig. 1B), whereas those obtained from RNP termini suggested two possible structures: one that might contain the polymerase complex (Fig. 1C) whereas the other could correspond to the closing loop (Fig. 1D).

Several classes from central sections of frozen-hydrated RNPs were composed of straight RNPs images, whereas other showed curved structures not amenable to further analysis. To perform the 3D reconstruction, we obtained an estimation of helical parameters from a symmetry-free refinement using images of straight RNPs and a featureless cylinder as starting model (17). Upon refinement using the iterative helical real space reconstruction protocol (IHRSR) (18), reconstructions of various classes converged to quasi-equivalent structures showing a dihedral helix with two opposite-polarity NP strands (Fig. 1E and fig. S2A). The double-helical structure defined a minor groove between the connected NP strands and a major groove between the NP strands not physically in contact. The helical parameters reached upon convergence of all structures (fig. S2B) were very similar: The rise step per monomer was constant at 28.4 Å, and the rotation angle between monomers ranged from -57° to -64° , forming helices with around 12 NP monomers per turn (six pairs). The basic features of the NP structure, including the head

and body domains, were visible in the helical structure and could be compared to the mini-RNP structure (15) (fig. S3). The docking of the NP atomic structure (13) into the helix places the head domain of each NP facing the major groove and the N-terminal regions contacting each other in the minor groove (Fig. 1, F and G). The excess density at the NP-NP interstrand connection could derive from the first 22 NP amino acids not represented in the crystal structure (fig. S4). For intrastrand NP-NP interaction, the 402-to-428 loop would be inserted into the neighboring NP in a way equivalent to that found in NP crystallographic structures (Fig. 1G). The high flexibility in the connections of the loop to the rest of the NP (fig. S5) allows the various dispositions among NP monomers observed in the helical RNPs, the crystal structures (12, 13), and the mini-RNP (15). Although the atomic structure of NP is conserved in the helical RNP and the distance between the loop and the body, 25 and 41 Å (Fig. 1G), is compatible with extended amino acid chains, some local rearrangements of the structure could not be ruled out.

To analyze the interstrand interaction, we compared the replication in vivo of a helical chloramphenicol acetyltransferase (CAT)-expressing RNP or a circular mini-RNP (15), in which the NP N terminus is not involved in NP-NP interaction. Because no atomic structure is available for the NP N terminus (12, 13), we assayed mutants lacking amino acids 2 to 8 (mutant N7) or 2 to 21 (mutant N20). Mutation N7 significantly reduced the accumulation of CAT RNPs but only slightly that of mini-RNPs, whereas mutation N20 strongly reduced replication of both (fig. S6). However, no gross alteration in the helical structure of recombinant RNPs containing N7 mutant NP was observed (fig. S6E). The relevance for intrastrand interaction of the critical residues at positions 412 and 416 at the swapping loop was also verified in both helical and circular RNPs (15) (fig. S6).

To determine the structure of the RNP termini, we performed a direct 3D classification of images from negative-stained samples using the maximum-likelihood procedures of Xmipp (19) and a filtered helical segment as initial model. The classification yielded six groups, two of which were assigned to the terminal loop and merged to generate the final structure (Fig. 2A). The electron density map showed a small loop where only three NPs are needed to connect the two helix strands (Fig. 2A, yellow). These results are consistent with the NP ability to form small, closed structures (9). Two other image groups showed the presence of the polymerase and revealed the existence of two conformations of the complex. The main structural difference is illustrated in gray (Fig. 2B) and suggested a rotation and displacement of the gray and green portions of the polymerase (Fig. 2B, diagram). The proportion of both conformations is roughly 50%, and their biological relevance is not clear. When compared to the polymerase in a mini-RNP (15), all structures showed the same appearance with a large central groove, but part of the polymerase is twisted and tilted in the helical RNPs in comparison with the mini-RNP (fig. S7, green). These structural changes are probably due to the minimal length of the mini-RNP template, which does not allow the formation of a helix.

The structure of the RNPs inside virions was determined by cryogenic electron tomography and subvolume averaging. The spikes, the envelope, and the inner M1 layer were visible as were individual RNPs in the internal density (Fig. 3A). RNP segments were selected and subjected to iterative alignment and averaging to improve resolution and to avoid the information loss resulting from the missing wedge. The helical parameters were determined in the averaged subvolume by using Xhelicals (18), and the values obtained at the minimum, a rotation angle of -64° and a rise step of 28.5 Å per monomer (fig. S8), were used to apply helical

Fig. 1. Structure of influenza virion RNPs. (A) Gallery of images of virion RNPs obtained from negative-stained samples. The blue and red squares illustrate the areas used for processing helical and terminal sections of the RNPs, respectively. (B) Two-dimensional average obtained from images of frozen-hydrated samples of RNP central regions (contrast inverted for comparison). (C and D) Two-dimensional averages of polymerase-containing and loop-containing RNP termini, respectively. (E) Three-dimensional cryo-EM reconstruction of the central portion of a virion RNP showing the two opposite-polarity NP strands in red and blue. (F) Docking of two NP monomers showing the interstrand connection. (G) Docking of two NP monomers showing the intrastrand connection. One of the monomers is shown in dark blue to illustrate the swapping loop and the distances of the flexible connections. Scale bar represents 100 Å in (B) to (D) and 50 Å in (E) to (G).

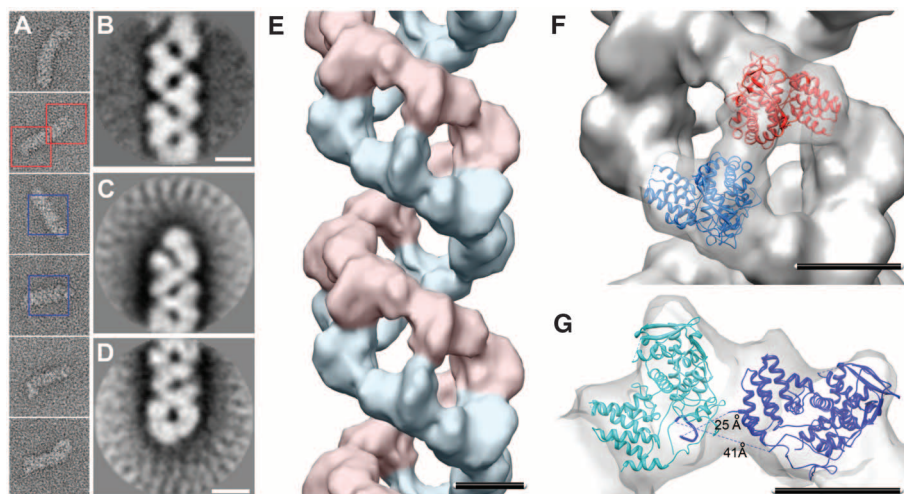


Fig. 2. Three-dimensional structure of the termini of influenza virion RNPs. (A) Structure of the terminal loop of a virion RNP juxtaposed to a portion of the helical section as a reference. The three NPs present in the loop are depicted in yellow, and the NP atomic structure is docked into one of them to serve as a size guide. (B) Structure of the polymerase-containing terminus of a virion RNP juxtaposed to a portion of the helical section as a reference. Two conformations of the polymerase are shown in top and bottom images, and the structural changes are outlined on the right.

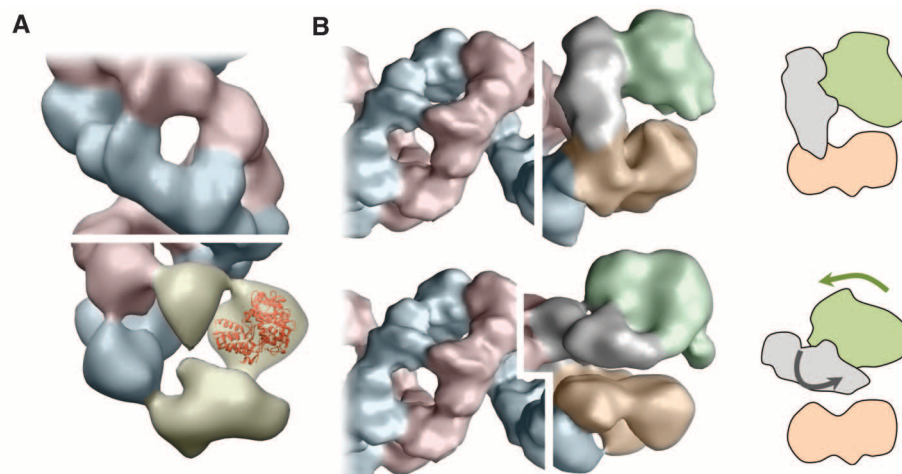
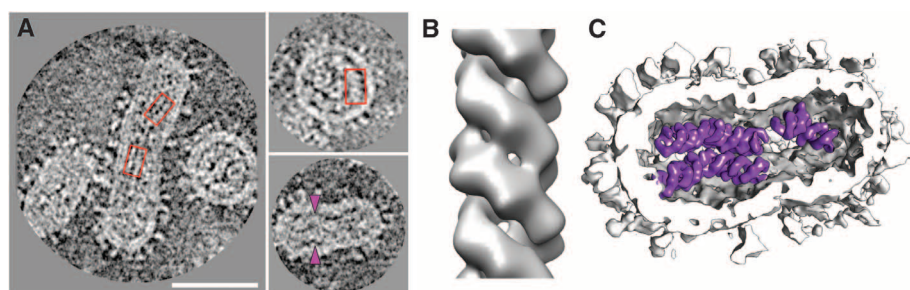


Fig. 3. Three-dimensional structure of influenza RNPs within intact virions. (A) Central sections of cryo-electron tomograms of frozen-hydrated A/WSN virions. RNPs are highlighted in red rectangles. (B) Averaged volume obtained after alignment and symmetrization of 288 volumes extracted from tomograms. (C) Volumetric representation of the segmented influenza virus showed in the bottom right image in (A). Averaged RNP volumes were placed back on the position and orientation computed in the alignment [densities marked with purple arrow heads in (A)]. Scale bar represents 1000 Å.



symmetry, although dihedral symmetry was not imposed (Fig. 3B). The symmetrized sub-volume was similar to soluble RNPs and showed a major and a minor groove, verifying that the structure of the RNPs inside virions is the same as the isolated ones. The final volumes were then placed back into the cryo-tomograms by using the coordinates and orientation of the helical axis (Fig. 3C), verifying their close interaction in bundles (20, 21).

By a combination of cryo-EM and cryo-electron tomography, we show the complete structure of native influenza virus RNPs. The composite model resulting from the juxtaposition of the polymerase-containing terminus, the helical internal portion, and the terminal loop portrays the most common of the quasi-equivalent structures present in the sample (Fig. 4A). Because this RNP structure derives from purified virions, it represents the incoming RNPs in the infected cell and the RNPs to be encapsidated in progeny virus. This RNP structure has implications for various steps in the infection cycle. After uncoating, parental RNPs are transported to the nucleus in a movement governed by two nuclear localization signal sites present in NP (22–24) whose locations in the RNP structure document their accessibility, in agreement with previous results (23, 25). The position of the RNA template was inferred from the surface potential of the docked NP monomers and the positions of mutations that reduce the RNA binding ca-

capacity of NP (12). The RNA could be located along the positively charged path present in each NP strand, as shown in alternative views of three NP monomers from the same strand (Fig. 4B and movie S1). The length of the RNA included into the path was around 140 Å per NP, in agreement with the 20 to 24 nucleotides per NP value previously estimated (7, 14, 16). Also, the RNP structure described here imposes constraints and suggests mechanisms for virus transcription and replication. The disposition of the RNA along the NP strand implies that the interstrand interaction should be disrupted for template copy, but the polymerase may only require local RNP destabilization because the template RNA is accessible to the solvent (26). The NP N terminus appears important for *in vivo* RNP replication (fig. S6) but not for *in vitro* transcription efficiency (fig. S9). These results are consistent with the trans/cis model for replication/transcription of virion RNPs (3, 27), which imply the replication of the template RNP by a nonresident polymerase while the resident polymerase would transcribe *in cis*. Mutation of the NP N terminus could affect recognition of the helical RNP or destabilize the binding of a nonresident polymerase, without affecting template accessibility for the resident polymerase during transcription. The progeny RNPs are assembled by protection of the nascent 5' RNA terminus by an additional polymerase and sequential incorporation of NPs (3, 27, 28),

but it is unclear how they would adopt the double-helical structure. One possibility is that the association of assembling and replicating polymerases during RNA synthesis (29) facilitates the 3'- and 5'-RNA termini interaction and induces a zippering reaction along the RNP. Moreover, the structure of helical RNPs suggests a potential mechanism for the generation of influenza defective-interfering particles. Because the NP strands are in close proximity along the structure, the replicating polymerase could easily switch strands and continue reading the template at a disparate position in its primary sequence, leading to internal-deletion defective RNPs (30).

Lastly, encapsidation into progeny virions is an ordered process, whereby the eight different RNPs associate to become enveloped at the plasma membrane, and relies on specific *cis* signals at the 5'- and 3'-proximal regions of each RNA (5, 6). According to the structure presented here, only a fraction of the RNA sequence would be exposed at the surface of the RNP, and the precise position of each *cis* signal would translate into a specific spatial disposition in the RNP that could determine its interaction with other(s) RNP(s). The combination of mutational data and the structure shown here will allow understanding the packaging code of influenza virus and will provide insight into how reassortment among influenza viruses of different subtypes occurs.

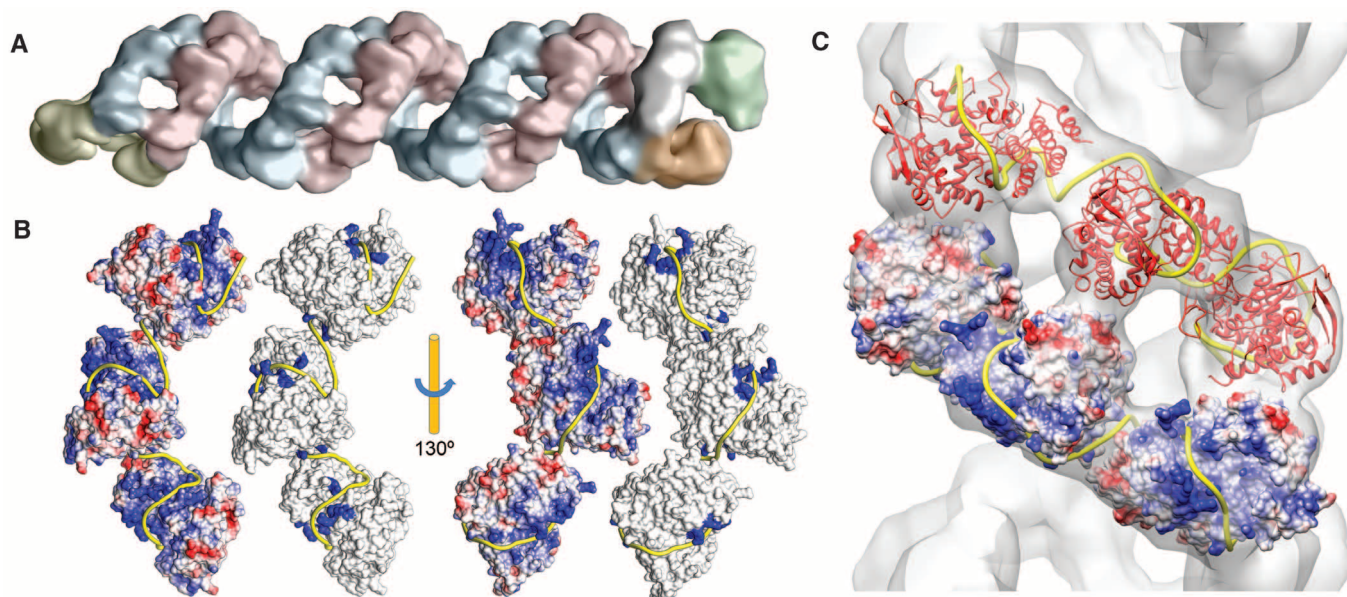


Fig. 4. Model for the structure of an influenza virus helical RNP. **(A)** Composite volume generated by combination of the central portion of an RNP and the RNP ends showing the polymerase (green and brown) and the terminal NP loop (yellow). This structure represents the complete model of the segment 8 RNP. **(B)** Model for the localization of the viral RNA (yellow thread). Four views

of the same NP strand are represented as surface potential and show, highlighted in blue, the residues whose mutation reduce NP-RNA interaction (12) **(C)** Model for the localization of the template RNA (yellow thread) in the helical structure of an RNP, showing one of the NP strands as surface potential and the opposite strand as a ribbon representation.

References and Notes

- G. Neumann, T. Noda, Y. Kawaoka, *Nature* **459**, 931 (2009).
- G. Neumann, G. G. Brownlee, E. Fodor, Y. Kawaoka, *Curr. Top. Microbiol. Immunol.* **283**, 121 (2004).
- P. Resa-Infante, N. Jorba, R. Coloma, J. Ortín, *RNA Biol.* **8**, 207 (2011).
- R. W. Ruigrok, T. Crépin, D. J. Hart, S. Cusack, *Curr. Opin. Struct. Biol.* **20**, 104 (2010).
- E. C. Hutchinson, J. C. von Kirchbach, J. R. Gog, P. Digard, *J. Gen. Virol.* **91**, 313 (2010).
- T. Noda, Y. Kawaoka, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 8797 (2012).
- R. W. Compans, J. Content, P. H. Duesberg, *J. Virol.* **10**, 795 (1972).
- M. W. Pons, I. T. Schulze, G. K. Hirst, *Virology* **39**, 250 (1969).
- R. W. Ruigrok, F. Baudin, *J. Gen. Virol.* **76**, 1009 (1995).
- K. Klumpp, R. W. Ruigrok, F. Baudin, *EMBO J.* **16**, 1248 (1997).
- K. G. Murti, R. G. Webster, I. M. Jones, *Virology* **164**, 562 (1988).
- A. K. Ng *et al.*, *FASEB J.* **22**, 3638 (2008).
- Q. Ye, R. M. Krug, Y. J. Tao, *Nature* **444**, 1078 (2006).
- J. Ortega *et al.*, *J. Virol.* **74**, 156 (2000).
- R. Coloma *et al.*, *PLoS Pathog.* **5**, e1000491 (2009).
- J. Martín-Benito *et al.*, *EMBO Rep.* **2**, 313 (2001).
- Information on materials and methods is available on Science Online.
- E. H. Egelman, *Ultramicroscopy* **85**, 225 (2000).
- S. H. Scheres *et al.*, *Nat. Methods* **4**, 27 (2007).
- T. Noda *et al.*, *Nature* **439**, 490 (2006).
- T. Noda *et al.*, *Nat. Commun.* **3**, 639 (2012).
- R. Bullido, P. Gómez-Puertas, C. Albo, A. Portela, *J. Gen. Virol.* **81**, 135 (2000).
- J. F. Cros, A. García-Sastre, P. Palese, *Traffic* **6**, 205 (2005).
- M. Ozawa *et al.*, *J. Virol.* **81**, 30 (2007).
- W. W. Wu, L. L. Weaver, N. Panté, *J. Mol. Biol.* **374**, 910 (2007).
- F. Baudin, C. Bach, S. Cusack, R. W. Ruigrok, *EMBO J.* **13**, 3158 (1994).
- N. Jorba, R. Coloma, J. Ortín, *PLoS Pathog.* **5**, e1000462 (2009).
- F. T. Vreede, T. E. Jung, G. G. Brownlee, *J. Virol.* **78**, 9568 (2004).
- N. Jorba, E. Area, J. Ortín, *J. Gen. Virol.* **89**, 520 (2008).
- P. A. Jennings, J. T. Finch, G. Winter, J. S. Robertson, *Cell* **34**, 619 (1983).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1228172/DC1
Materials and Methods

Figs. S1 to S9

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Movie S1

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New Products



REFRIGERATED INCUBATOR

The new KT 115 refrigerated incubator utilizes energy-efficient, state-of-the-art Peltier refrigeration technology to provide secure, accurate control. With comprehensive program functions, the unit offers a wide range of capabilities for incubating biological materials and guarantees reproducible results. Functional chamber volume is 3.7 ft³ (104 L), and the unit can run at temperatures down to 4°C. In contrast to a compressor, the Peltier technology, which is used exclusively for cooling, is vibration free and significantly reduces energy consumption. Conventional heating technology extends the operating life of the device, which can be conveniently decontaminated at its 100°C maximum temperature. Proven APT.line preheating chamber technology ensures homogenous temperature conditions, as well as rapid heating and recovery times. Horizontal airflow from both sides of the chamber provides uniform temperature distribution, even when the unit is fully loaded. Temperature, set digitally, is accurate to a tenth of a degree and ensures effective and gentle incubation.

Binder

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3-D IMAGING SYSTEM

The Lightsheet Z.1 provides biologists with a new method of imaging dynamic processes in living organisms. Biologists can use the new microscopy system to observe the development of entire organisms over several days or more. The extremely low phototoxicity and the integrated incubation enable gentle imaging of specimens over hours to days. Lightsheet Z.1 works with an expanded light beam (the light sheet) that illuminates only a thin section of the sample, thus protecting the rest of the specimen. Images are captured at a 90° angle to the light sheet. Multiview imaging allows data acquisition from different viewing angles. These can be combined through mathematical algorithms into 3-D reconstructions and time-lapse videos. The light sheet system of the Lightsheet Z.1 uses a new type of optical concept that combines cylindrical lens optics with laser scanning. Users receive homogeneously illuminated optical sections of complete examination objects.

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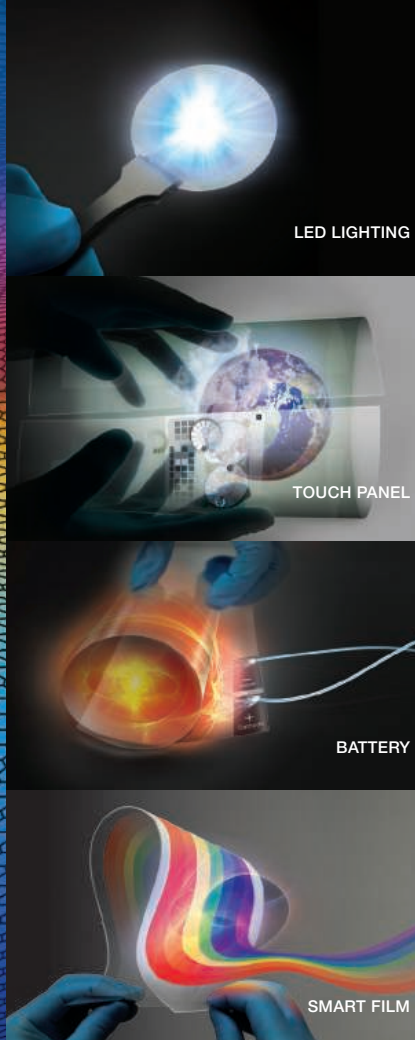
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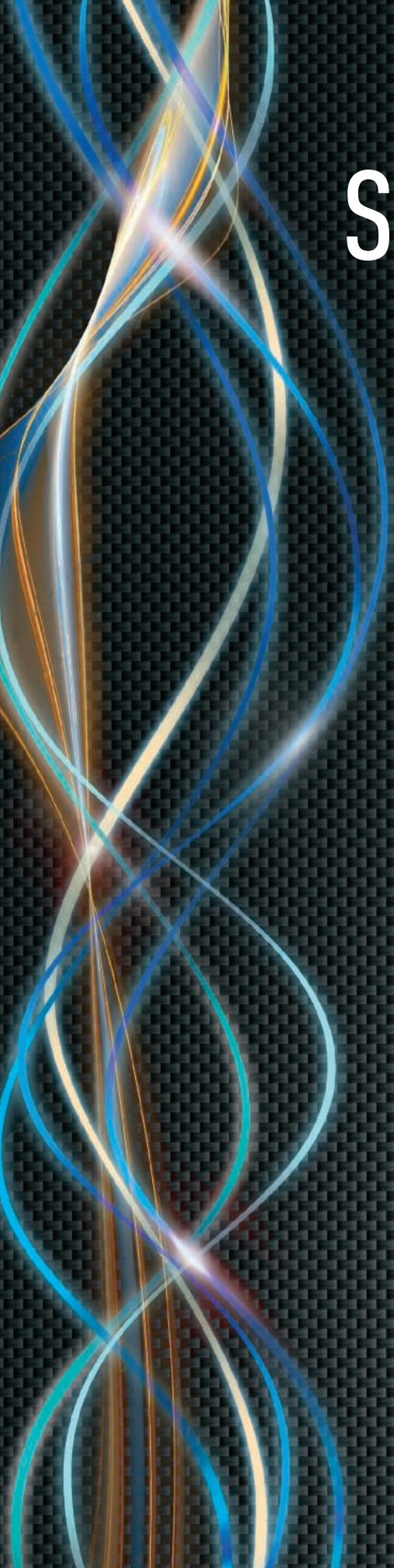
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Tianfu Life Science Park: Gem in the Hub of Chengdu Bioscience Cluster

Chengdu City: A blend of ancient culture and modern civilization

The life science and health industry of China will maintain rapid growth in the next decades, as in 2009 China announced a CNY 850 billion (US\$124 billion) stimulus package to fundamentally reshape the nation's healthcare sector, including the expansion of health service to rural areas in Western China and the mobilization of substantial resources to foster growth of the industry. In the midst of these developments, Tianfu Life Science Park can be found nestled in the Chengdu High-Tech Zone. Companies located in the zone benefit from a broad base of support mechanisms designed to promote innovation and growth.

Built 2,300 years ago, Chengdu, the capital city of Sichuan Province, is known as the Land of Heaven owing to the fertile Chengdu plain and favorable growing conditions. Abundant harvests are characteristic of the region and have been for millennia, thanks to a feat of ancient engineering. The irrigation structure known as the Dujiangyan, built in 256 BC, protects Chengdu from extreme conditions such as drought or flooding. Today Dujiangyan dams provide irrigation for over 5,300 km² of land in the region. 'Tianfu' means 'paradise' to the locals, and the Tianfu Life Science Park serves as the city's important innovation and incubation center for biomedical research and development.

A Booming Cluster with Real Substance

Chengdu and its large life science market are characterized by their outstanding level of biomedical research and valuable discoveries within the life sciences. A number of Chengdu companies that took advantage of this strong atmosphere and abundant resources for research and development to create and commercialize products, have truly succeeded in the market. In line with the general increase in support for biotech, Chengdu aims to continue to be the center of a booming cluster of biotechnological innovations, enterprises and collaborations.

Chengdu is the well-connected hub for biopharmaceutical industry in Western China, home to almost 500 enterprises specialized in modern traditional Chinese medicine, chemical drug, biopharmacy, medical appliances, medical packaging materials, pharmaceutical research and development services, etc., including 209

industrial enterprises with an annual main business revenue over CNY 20 million each, and 90 enterprises with an annual sales revenue over CNY 100 million each, and its gross industrial output ranks first in west china. In 2011, biopharmaceutical industry above designated size achieved a sales revenue of CNY 31.091 billion with an added value of CNY 12.543 billion and had complete industrial cooperation and support capability.

The natural resources located in and around Chengdu are a source of over 2,000 types of Chinese herbal medicines, which account for approximately one-half and one-third of those marketed in Sichuan Province and China, respectively. Not surprisingly, Chengdu is known as the 'hometown of traditional Chinese medicine' or the 'traditional Chinese drug warehouse'. The area dedicated to planting traditional Chinese medicinal materials, such as chuanxiong rhizome, curcuma root, Chinese goldthread and magnolia bark, is maintained at or above 400,000 mu (about 266,660 m²) throughout the year.

Chengdu's global position in other technology sectors means the logistics of travel and export are well established. The city has an excellent domestic sea, railway and road transport and logistics system. Chengdu is one of China's four major international airport hubs, with the largest airport in Midwest China and 34 international airways. The railway-sea combined transport logistics thoroughfare connects European, Middle East and Southeast markets via the Euro-Asian Continental Bridge and the Pan-Asian Railway Line. In 2009, the comprehensive clearance capability of Chengdu customs ranked sixth among China's 41 customs offices and first in Midwest China.

Chengdu is regarded as a metropolitan area for bioscience employment. In 2009, the area boasted 42 general colleges and universities, more than 1,000 scientific research institutes, 589,000 university and college students and 146,000 graduates. Ten universities offer a major in pharmaceutical sciences, and 13 offer a major in chemistry. 12 vocational and technical colleges offer secondary technical training in pharmaceuticals. Nearly 10,000 professionals in various fields are trained each year. Chengdu has the world's largest clinical education training center, which is certified by the American College of Surgeons in Asia. Compared with other major cities of China, Chengdu has the additional advantage of being able to provide a talent pool at a lower human resource cost than can cities in coastal areas.

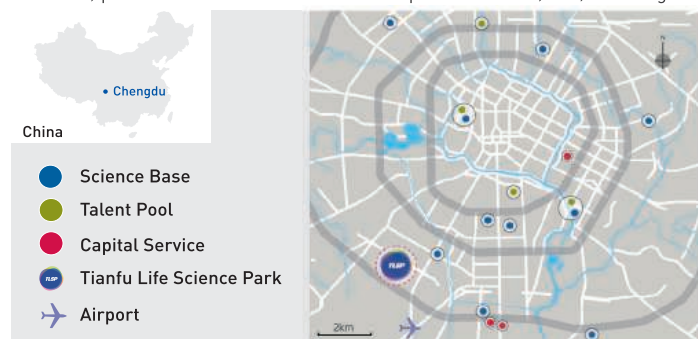
Well Established Life Science Industry Network

Universally, successful biopharmaceutical clusters encompass three elements: universities to drive innovation and train a scientific workforce, financing to support companies founded on innovation and, of course, laboratories and space for businesses to grow. Chengdu possesses all the elements necessary to support a burgeoning biomedical cluster and which all form a well established life science industry network.

Now Chengdu has 64 colleges and universities and scientific research institutes, 8 national and provincial laboratories, 16 national and provincial engineers [technology]



Sichuan University (West China Campus)





BioTianfu Forum



Inside TLSP

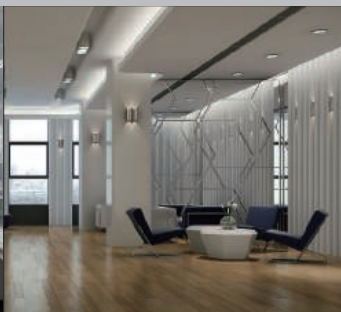


Dr. Li Jin

Dr. Chen Yuanwei



TLSP Public Laboratories



TLSP Incubator



Tianfu Life Science Park (TLSP)

research centers and enterprise technology centers, more than 100 research and development-oriented enterprises, etc. And there are 5 national bases for clinical trial of new drugs and 2 national centers for safety evaluation of new drugs. Chengdu has the largest medical laboratory center in China which is the only domestic center that has passed inspection certification of the College of American Pathologists (CAP) as a whole.

The government provides incentive by offering tax advantages, favorable development & investment promotion policies and financial support to life science industry. The government sets up research and development incubation fund for new drugs, business startup investment guidance funds, startup investment compensation funds, innovation and entrepreneurial seed funding and special funds for scientific and technological achievements transformation, supporting biomedical innovation and entrepreneurship and achievements transformation and implements biomedical science and technology industrialization projects to support new drug development and major scientific and technological achievements transformation.

And Chengdu High-Tech Investment Group and other financial institutions are providing capital investment through various financial mechanisms. Regarding space for businesses to grow, Tianfu Life Science Park, served as the city's life scientific and technological center, provides public laboratory space and business incubation facilities for biopharmaceutical SMEs.

Tianfu Life Science Park: Gateway of Life Science Industry in Western China

Set among the sculptured green spaces of Chengdu High-Tech Zone which ranks fourth among the 55 High-Tech Zones in China, the Tianfu Life Science Park occupies 221,553 m² of land. As the gateway of the life science industry in Western China, TLSP is an important innovation and incubation center for biological and medical research and development, and serves as a platform for the cooperation between medical/clinical institutions at home and abroad.

In TLSP, in compliance with the requirements of BioSafety level 2 (P2), the construction of 6 independent public laboratories has been basically completed, namely, analysis lab, molecular lab, cell culture lab, separation & purification lab, natural medicine lab, and general lab, have a total GFA 1731 m² and 63 sets of large, state-of-the-art equipment and can satisfy the R&D needs in the fields of, among others, molecular biology, immunology, genetic engineering, genomics, proteomics, transgenic animal/plant cell culture, microbial culture, natural products extraction and separation, drug synthesis and analysis. More, there is additional space about 1501 m² available for any joint laboratories. In the Park, many a fully-furnished incubator unit has been basically completed, ranging from 135 to 315 m² and totaling 18,746 m² (GFA). Consisting of its own functional area (lab, office space, equipment room, records room, storage room, etc.), each unit will be an independent laboratory with full office and laboratory features.

The leading-edge offices, laboratories and facilities in TLSP enable scientists, technologists and enterprises to save development costs and time to market. They can nurture ideas, innovate and develop through the dynamic environment provided by TLSP and also expand their business opportunities by strengthening collaboration with the growing network of partners.

We Choose TLSP

TLSP is home of more than 70 well-known companies and institutions engaging in biotechnology and ranging from start-up, SME to conglomerate.

West China Hospital, Sichuan University : West China Hospital has been ranked among the top hospitals in China since 1990 and is the medical center of sophisticated and severe cases, the center of medical education and the scientific research in Southwest China arranged by MOH and MOE.



HitGen: HitGen aims to establish a unique platform and associated capabilities for drug discovery research. HitGen will build a core platform for progressing projects from targets to the leads stage, including high quality and large compound libraries (encoded and discrete), screening and selection, and informatics analysis and design.



Genekey Biotech: Genekey Biotech focuses on Biotechnology in drug research and development. One researching new drug is a B7.1 fusion protein consisting of the extracellular domains of human B7.1 and the Fc portion of human IgG1, called B7.1-Fc.



ChemPartner: ChemPartner is a wholly owned subsidiary of ShangPharma Group, a leading China-based pharmaceutical and biotech R&D outsourcing company (CRO) listed on NYSE with symbol SHP. ChemPartner has established a state-of-the-art drug discovery service platform including 70,000 sq ft research lab and modern analytic instruments.



Origissay Diagnostics Ltd: Chengdu Origissay Diagnostics, Ltd. specializes in research, development, manufacture and marketing of in-vitro diagnostic products. The company's patented product Rapid Diagnostic Kit for PROM-LEAKECTION™ is being sold on Chinese markets. Its diagnostic accuracy has reached 97% or more. The company has entered into an intent of cooperation with Maastricht University in the Netherlands to sell the product on European markets.



MedGene: MedGene Biopharm(Chengdu) Co., Ltd, collaborating with University of Toronto, is a biotechnology company to develop novel drugs and approaches targeting cancer stem cells(CSCs) which may cure cancers.



BioTianfu: The Voice of Chengdu's Bioscience Industry

The BioTianfu is a platform where life science industry participants across the entire value chain gather. TLSP is one of the pioneers of the BioTianfu and it is created mainly for integrating innovative resources within the region and pushing the industry forward. The BioTianfu enables experience sharing through customized interactive lectures sessions, panels and roundtable meetings. The BioTianfu has been held twice successfully since it was initiated in 2011 and is getting a solid reputation.



Tianfu
Life Science Park™
天府生命科技园

CONTACT DETAILS

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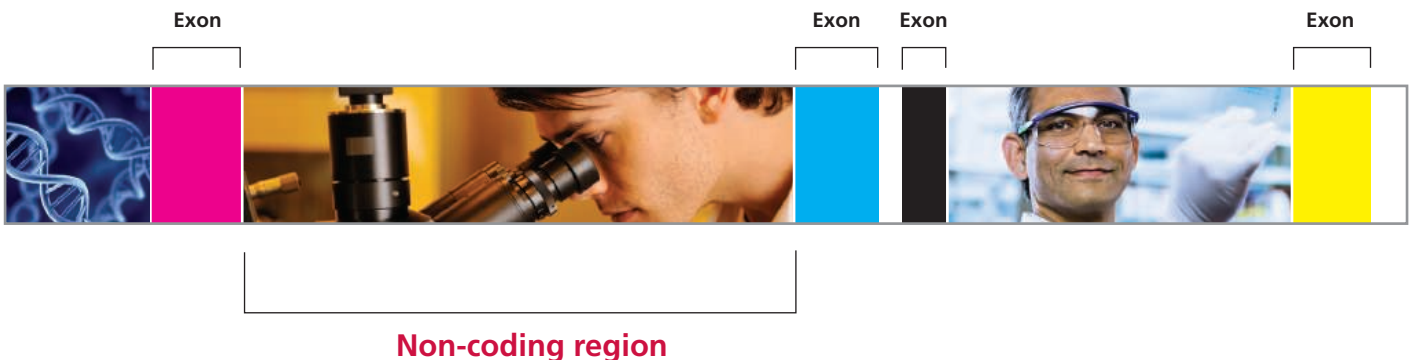


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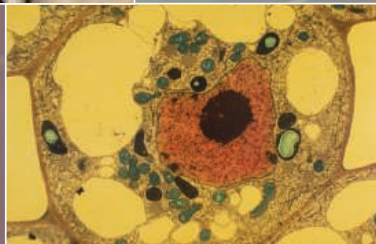
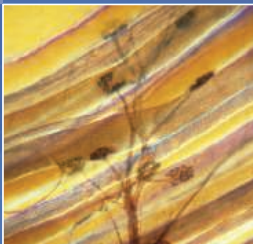
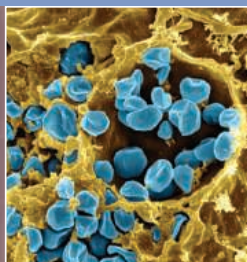
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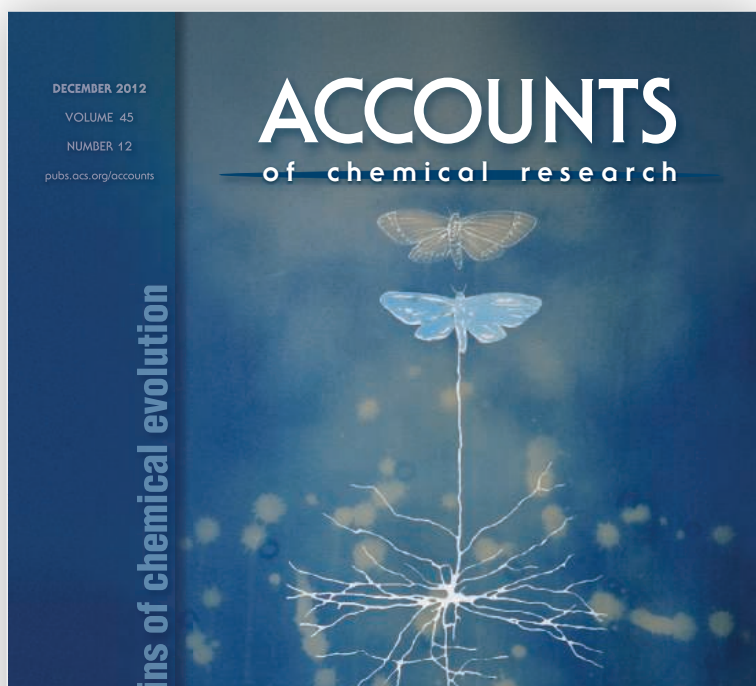
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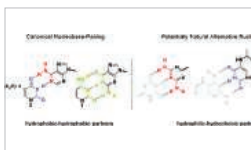
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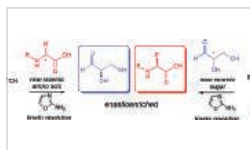
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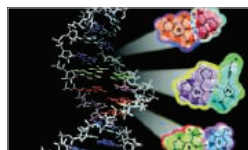
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Steven A. Benner, Hyo-Joong Kim, and Matthew A. Carrigan
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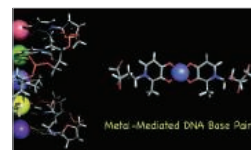
Role of pK_a of Nucleobases in the Origins of Chemical Evolution
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Natural versus Artificial Creation of Base Pairs in DNA: Origin of Nucleobases from the Perspectives of Unnatural Base Pair Studies
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Sincerely,

A handwritten signature in blue ink, appearing to read 'Ian King'.

Ian King
Director of Marketing and Membership, AAAS



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HONG KONG IN FOCUS: Asia's Research Hub



Positioned at the crossroads of Asia and the West, both geographically and culturally, Hong Kong is taking advantage of its auspicious place in Asia by reimagining itself not just as a financial and tourism center, but also as a research and biotechnology hub. This sponsored feature will provide the reader with an update on the state of scientific research in the region and a glimpse into the powerful engine that is driving progress there, from basic research to the commercialization of therapeutics and medical devices.

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Overview

Hong Kong is a special administrative region of China with a population of seven million. As a former British colony, handed back to China in 1997, Hong Kong still contains a unique blend of East and West. Its educated, multilingual populace speaks Cantonese and Mandarin,

while English continues to be the language used in business, education, and research and development. This makes Hong Kong an attractive place for international talent and a bridge between Asia and the rest of the world.

Hong Kong is best known as a financial center and the business capital of Asia. However, biotech is an emerging sector that has been designated a key industry for development.

"We have to focus on developing a key area we identify to have huge potential, and grow it to assume a leading position globally. It is well-recognized that Hong Kong possesses what it takes to become a high-tech hub, and we are hoping to unify the efforts of the government, up-stream academia, and mid- to downstream industry," says The Honorable Chun-Ying Leung, chief executive of Hong Kong, in a recent interview with the Hong Kong-based newspaper, Wenweipo. The Hong Kong Government is determined to promote biotech as a key industry.

Six key industries have been developed and intensively promoted, of which four are closely related to biotech, namely innovation and technology, testing and certification, medical services, and environmental industries. The remaining two areas are educational services and creative industries.

Hong Kong Science and Technology Parks Corporation (HKSTPC) exemplifies what can be achieved under this paradigm. "The advantages of performing biotech research and development in Hong Kong are myriad.

Under the 'one country, two systems' doctrine, Hong Kong has protected intellectual property [IP], internationally recognized enforcement of common law, free media, academic freedom, and a sound financial and banking system which can provide a platform for fundraising. Hong Kong Science Park provides the infrastructure, support programs, and collaboration opportunities to nurture the growth of our biotech industry and to support Hong Kong to grow into a knowledge-based economy," says Mr. Nicholas Brooke, chairman of HKSTPC. Their 22 hectare Hong Kong Science Park currently provides laboratory and office space to almost 400 technology companies.

Today, there are 250–300 biotechnology companies in Hong Kong,

70 with substantial mainland background. Hong Kong's total R&D expenditure recently more than doubled from HK\$5.9 billion (US\$761 million) in 1999 to HK\$13.3 billion (US\$1.7 billion) in 2010. The number of full time R&D employees also doubled, from around 10,000 to 24,100 during the same period. Government support also comes in the form of the Innovation and Technology Fund (ITF), set up in 1999, which aims to support mainly applied R&D projects conducted by universities, industry support organizations, industry and trade associations, and private sector companies that contribute to the innovation and technology industry. "As of October 2012, HK\$7.1 billion (US\$916 million) of ITF funding has been approved for 3,066 projects, of which around seven percent went into biotechnology-related applications," says Miss Janet Wing-Chen Wong, the commissioner for Innovation and Technology.

Professor Albert Cheung-Hoi Yu, chairman of the Hong Kong Biotechnology Organization (HKBIO), emphasizes that this special feature outlining Hong Kong's biotech capabilities is a strong signal to the international community of where the city is heading. "A number of very

active, fast-growing biotech players from the government, academia, and industry are featured. But this is not an exhaustive representation of what Hong Kong has." Examples of other crucial components of Hong Kong's biotech

include the Hong Kong Medical and Healthcare Device Industries Association, The Hong Kong Association of the Pharmaceutical Industry, Hong Kong Business Angel Network, and the quasi-government run Hong Kong Productivity Council, amongst others. Education-wise The Open University of Hong Kong and the Vocational Training Council are, among other higher and continuing education institutes, supplying biotech talent in Hong Kong. "Many other outstanding professors and researchers also contribute significantly to Hong Kong's biotech advancement into a globally outstanding research hub," added Professor Yu.

"With world-class universities, Hong Kong is strong in basic research and making progress in the nurturing of industry. Its unique geographical and political position, and close economic ties to the mainland, allows its biotech industry to benefit from China's R&D drive," says The Honorable Regina Suk-Yee Ip Lau, honorary advisor of HKBIO and member of the Executive Council, Government of the Hong Kong SAR. Indeed, Hong Kong is rapidly becoming the chosen location for both global companies who want to access the fast-growing market in mainland China and for Chinese companies wanting to reach the rest of the world.

It is well-recognized that Hong Kong possesses what it takes to become a high-tech hub.

-The Honorable Chun-Ying Leung



Chun-Ying Leung



Nicholas Brooke



Regina Suk-Yee Ip Lau

Innovation and Technology Commission www.itc.gov.hk

The Innovation and Technology Commission (ITC) was set up in 2000 to enhance Hong Kong's innovation and technology capability as an impetus for economic growth by supporting applied research and development, and technology transfer and application, providing technological infrastructure, and nurturing the development of human capital.

"Our basic policy is to create an ecosystem within which the innovation and technology sector can survive and flourish," explains Miss Wong.

The attraction of Hong Kong according to Miss Wong are its geographical location in Asia, the use of the common law system, the simple and affordable tax system, a clean government, and credible IP protection. Additionally, the region has universities that persistently rank among the top 50 in the world.

Importantly, the living environment of Hong Kong in terms of language, mobility, convenience, and familiarity has created a culture attractive to people from all over the world. "It is a good place for foreigners to connect with businesses in the mainland and Asia," says Miss Wong. "For big

Chinese companies, Hong Kong is a good place to work towards the international market. We are really right in the heart of Asia."

Despite this, biotech in Hong Kong is a relatively young technology sector. However, health care-related companies, such as pharmaceuticals, medical devices, diagnostics, and traditional Chinese medicine, are growing. "Modernization of traditional Chinese Medicine [TCM] is increasingly showing huge potential and provides the perfect East-West integration that is crucial for Hong Kong's portfolio," adds Miss Wong.

"For biotech, proximity to the mainland provides oppor-

tunities. Although Hong Kong is relatively small, it shouldn't be considered in isolation," states Miss Wong. Hong Kong is partnered with 12 of the 260 State Key Laboratories in the mainland China (with more to come), 10 of which have a biotech-related research focus. ITC, in collaboration with the Research Grants Council, ensures that these laboratories meet international standards with regards to infrastructure, workforce, and research focus.

Miss Wong expects biotech in Hong Kong to flourish and develop in the future. "We are certain that more international partners, including academics and companies, will join us in the future," she says.

"Our basic policy is to create an **ecosystem** within which the innovation and technology sector can survive and **flourish**."

-Miss Janet Wing-Chen Wong

Hong Kong Biotechnology Organization www.hkbio.org.hk

Hong Kong Biotechnology Organization (HKBIO), a charitable biotech industry organization, was established with the objectives of advancing and accelerating Hong Kong's biotech industry growth by promoting study, research, education, and exchanges in the biotech sector in Hong Kong. "Building an industry requires concerted effort from government, academia, and industry," explains Professor Yu. "We want to show the international community that Hong Kong is fully committed to building and growing this industry and that our government, academia, and industry are all taking proactive roles in contributing to biotech."



Albert Cheung-Hoi Yu

"We want to show the international community that Hong Kong is **fully committed** to building and growing this [biotech] industry."

-Professor Albert Cheung-Hoi Yu

"HKBIO has extensive connections worldwide, bringing together local companies and potential global partners," says Dr. Bernard Pak-Li Chan, HKBIO Council Member. Some examples include the signing of memoranda of understanding with

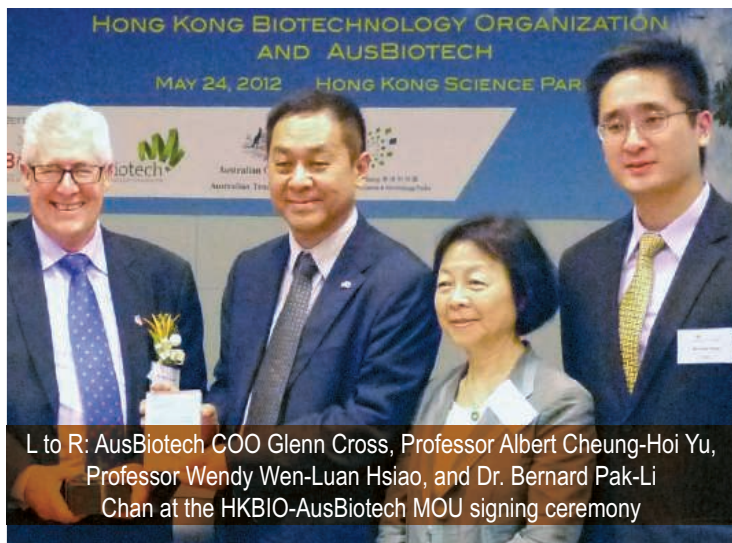
the Taiwan Bio Industry Organization and AusBiotech, and the various delegations to build global platforms for industry.

"In general it is hard to attract funding specific for the biotech industry, so part of HKBIO's mission is to bring in venture capitalists and other funding agents to our industry, so that they will have opportunities to match each other's needs and resources," says Professor Wendy Wen-Luan Hsiao, HKBIO treasurer.

"One of the initiatives is to assist our industry to explore the China market. Hong Kong is the gateway to China," says Professor Yuk-Lam Lo, HKBIO honorary chairman. "It is very easy for us to go into China to do business and utilize the resources, including talent, financial grants, and the future market," adds Professor Yu. "In China, the demand for medical care, medical diagnosis, and treatments will be huge. The market in America is already saturated, but in China, you cannot calculate how much it might be worth."



Janet Wing-Chen Wong



L to R: AusBiotech COO Glenn Cross, Professor Albert Cheung-Hoi Yu, Professor Wendy Wen-Luan Hsiao, and Dr. Bernard Pak-Li Chan at the HKBIO-AusBiotech MOU signing ceremony

Hong Kong Science and Technology Parks Corporation

www.hkstp.org

Located at the waterfront of Tolo Harbor adjacent to The Chinese University of Hong Kong in the New Territories, the 22-hectare Hong Kong Science Park, which is managed by Hong Kong Science and Technology Parks Corporation (HKSTPC), was constructed to attract technology firms to base operations and R&D in Hong Kong. Phase 1 of the Park was opened in 2002 with Phase 3 due to be completed in stages from the end of 2013 to 2016.

“Our vision is to help Hong Kong build its capability in technology and innovation development,” explains Mr. E. Anthony Tan, chief executive officer of HKSTPC. “And to turn discoveries and new inventions from an idea or concept into a commercial product or service.”

To enable this, HKSTPC offers state-of-the-art infrastructure and offices for applied R&D activities, and shared laboratories with technical support to help reduce capital investment of R&D companies in product design and development. The five major technology clusters in the Park include electronics, information technology and telecommunications, precision engineering, biotechnology, and green technology. Around 55 of the 400 companies based in the Park work in biotech, employing roughly 800 people. The Park is currently 95 percent occupied; Phase 3 will provide another 50 percent capacity.

Gathering like-minded industries together was crucial to the Park’s design. “One important element of the Park is the clustering effect,” says Mr. Tan. “By focusing on certain technologies we can pool the right companies together who can create new ideas and challenge each other.

Among the five clusters, biotech is one of the important sectors for the 21st century,” adds Mr. Tan. “Many of the the issues in health and life sciences depend on biotech to provide solutions.”

“Our vision is to help Hong Kong build its capability in technology and innovation development.”

-Mr. E. Anthony Tan

“We provide a platform to facilitate: we support mature and medium sized companies,” explains Mr. Young. “More importantly, one of our main focuses is to support the startups, nurturing them and helping them with financial and non-financial support.”

HKSTPC’s incubation programs provide not only low-cost accommodation, but also management, marketing, financial, and technical assistance during the crucial first two to four years in the lives of startup companies. Incubatees can apply for a financial aid package worth up to HK\$860,000 (US\$112,000) over a four-year period. Beyond this, HKSTPC also implements a small and medium-sized enterprise program which helps companies with financial and legal services, office space, shared facilities, and more.

Beyond this, Hong Kong Science Park is an important new part of Hong Kong’s infrastructure as it turns itself into a regional hub for innovation and technology. “Many think of Hong Kong as a tourist spot, a trading center, and a logistics center. But not yet as a technology center. So we really need to focus on making a difference at the Park. We want to show the world that Hong Kong is capable of excelling in developing selected technologies,” explains Mr. Tan.

Mr. Young believes that the Park will be part of the community. “We serve the local community and also act as a platform for overseas technology companies to capture the Asia market,” he explains. “We also enable Chinese technology companies to use Hong Kong as a base for their own internationalization.”

Moving beyond Hong Kong itself, HKSTPC works closely with Chinese authorities at the national and provincial levels, as well as with a technology park in Guangzhou, mainland China. HKSTPC is also currently improving shipping methods on both sides of the Hong Kong-China border to allow for the smoother exchange of samples between R&D laboratories.



E. Anthony Tan



Andrew Meng-Cheung Young



Hong Kong Science Park Phase 3

"It's very important to collaborate with China," agrees Mr. Tan. "It's an area from which we can draw a lot of resources in terms of technicians, engineers, and scientists. We can also leverage Hong Kong's ability to attract the best and brightest from around the world. HKSTPC can help develop breakthrough technologies using Hong Kong's unique environment."

Mr. Young is looking towards a bright future for HKSTPC. "Hong Kong is not yet known as a place for science and technology development. But some very innovative technology developers and entrepreneurs are willing to take the plunge. Hong Kong is much more than a physical location: it is a living community—that is what Hong Kong Science Park is all about."

Multigene Diagnostics, Ltd.

www.multigene.com.hk

Multigene Diagnostics, Ltd. (Multigene) is a spin off from the City University in Hong Kong and the first biotech company admitted into the Incu-Bio Business Incubation Program of HKSTPC in 2009. The company is developing a range of molecular diagnostic assays using multiplexed fluorescence and array-based platforms for detection of infectious pathogens and genetic diseases, and for early screening and mutation typing of cancers.



Lawrence Chi-Hung Tzang

"We are in a unique position to rapidly convert biomedical research into molecular diagnostic products for commercialization," states Dr. Lawrence Chi-Hung Tzang, executive director of Multigene. "We are collaborating with universities in Hong Kong and mainland China, where the government has significantly increased the investment in research and development in recent years." In addition to its R&D capability, Multigene has established an ISO15189:2007 accredited medical laboratory in Hong Kong Science Park, the first in the private sector in Hong Kong. "All our products are validated and offered as clinical testing services to local physicians and diagnostic laboratories. This will significantly shorten the time it takes when the products go through clinical trials and regulatory processes of SFDA [China's State Food and Drug Administration] required for entering the mainland market," explains Dr. Tzang.

The rapidly growing Chinese economy and the increased purchasing power of the population, coupled with an increased awareness of wellness and preventive medicine, and the desire for cutting-edge products and services, make the future outlook extremely positive for molecular diagnostics in the China market. "We have a number of products that are in great demand in China, such as the human papillomavirus (HPV) detection and genotyping kits for cervical cancer screening," says Dr. Tzang. "The other products that are ready for market include a multiplexed detection kit for 10 common sexually transmitted disease pathogens, an ovarian cancer screening kit, and a series of mutation

detection kits for personalized cancer therapy." Multigene plans to set up a manufacturing unit within a year and establish a marketing and sales network in mainland China.

Bio-Cancer Treatment International, Ltd. www.bio-cancer.org

Bio-Cancer Treatment International, Ltd. (BCT), a local biotech startup, was established in 2001. Dr. Paul Ning-Man Cheng, CEO of BCT led the project to develop pegylated recombinant human arginase (BCT-100), together with Polytechnic University's (PolyU) Professor Yun-Chung Leung and Associate Professor Dr. Wai-Hung Lo.



Paul Ning-Man Cheng

"BCT tests the hypothesis that human recombinant hepatic arginase I, after suitable pegylation to lengthen its circulatory half-life, can safely deplete plasma arginine for a prolonged period of time," explains Dr. Cheng. "Once this state of arginine depletion is achieved, we test whether it induces remission in certain cancers that are auxotrophic for the amino acid arginine." The goal is to provide a cancer treatment that is free of the side-effects usually associated with cancer chemotherapy.

Dr. Cheng believes that BCT's research is particularly relevant in China. Their main target is liver cancer, which is very prevalent in the region. "Over 45 percent of the world's liver cancer cases are found in China and Southeast Asia," says Dr. Cheng, whose project was incubated at PolyU with funding from a private venture capitalist group and Hong Kong's Innovation and Technology Commission.

Following optimistic preclinical results, BCT plans to commercialize the new drug. Phase 1 clinical trials for liver cancer treatment have been completed, with phase 2 starting this year alongside phase 1 trials for refractory lymphomas and leukemia.

"This is the first homegrown drug that has gone through the United States' Food and Drug Administration process and is cleared for use on humans in the U.S. and Hong Kong," says Dr. Cheng. "We have good clinical data and, most importantly, there are no adverse side effects." This is an achievement which has been hailed as an important milestone in the development of the biotechnology and pharmaceutical industry in Hong Kong.

Lee's Pharmaceutical Holdings, Ltd. www.leespharm.com

Lee's Pharmaceutical Holdings, Ltd. (Lee's Pharm), is a public biopharmaceutical company that has operated for over 18 years in China, carrying out drug development and clinical research, as well as regulatory, manufacturing, sales, and marketing activities. Currently, 12 of its products are marketed in mainland China, with 30 more under development. The company's focus includes cardiovascular and infectious diseases, dermatology, oncology, gynecology, and ophthalmology, among others.

One product that Lee's Pharm's researchers are excited about is Yal-laferon®, a topical interferon. "Interferon is an effective antiviral agent produced by our own bodies but, being a protein, is unstable outside of the body," explains Dr. Benjamin Xiao-Yi Li, CEO of Lee's Pharm. "Our technology allows the protein to be stable at temperatures up to 20°C, suitable for topical application. Clinical studies have indicated its suc-

cessful use for genital herpes, genital wart, herpes zoster, and cervicitis. A recent study showed eradication of high-risk HPV infection in some patients. Since HPV infection can lead to cervical cancer and has become an important women's health issue in China, we are participating in a study conducted by the Chinese Ministry of Health to advance HPV prevention and treatment."



Benjamin Xiao-Yi Li

Another promising treatment is the anti-platelet drug, Anfibatide, which has been 14 years in development. "It is a glycoprotein 1b antagonist with much lower bleeding risk," explains Dr. Li. "This is particular relevant in China because our diet makes native Chinese more prone to bleeding. Anfibatide is the first product with a novel mechanism of action to be advanced to phase 2 clinical studies." Currently, the drug is being tested for treatment of acute ischemic cardiac syndrome, with results expected by late 2013.

High-quality research and development has led to Lee's Pharm being ranked second in the "Best Small-Cap Company in China" ratings by Finance Asia and to be included on the Forbes Asia's 200 Best Under A Billion companies list.

"Hong Kong has easy access to [the Chinese and Japanese] markets... and is a good location for partnerships with key opinion leaders from the region."

-Mr. Paul Young

Hologic, Inc. www.hologic.com

Hologic, known as "The Women's Health Company," concentrates solely on women's health care needs including osteoporosis assessment, HPV testing, fetal fibronectin tests, breast magnetic resonance imaging solutions, and improved Pap Tests, among others. "The most important area for us is breast health—breast cancer screening, diagnosis, and

treatment," explains Mr. Paul Young, Vice President of Hologic and General Manager of Asia Pacific. Hologic has created breast cancer screening products specifically for the Asian population through research collaborations with Hong Kong healthcare professionals.

While Hologic's world headquarters is in Boston, its Hong Kong branch in



Paul Young

Hong Kong Science Park acts as its Asia headquarters. Hong Kong was chosen because it is the "closet to the biggest markets in Asia: China and Japan," explains Mr. Young. "It has easy access to both markets, provides easy shipping in and out of Asia, and is a good location for partnerships with key opinion leaders from the region."

After careful consideration, Hologic chose Hong Kong Science Park for both its facilities and accessibility to other countries. "Also, there are many similar companies in the Park that we can talk to, making it easy to find commercial partners," says Mr. Young.

Hologic plans to expand into other areas, including men's health by forming key partnerships with universities in both China and Hong Kong. "We work with universities and university hospitals directly because they are our customers," explains Mr. Young. "And with the Chinese Society of Radiology on education in breast cancer screening and diagnosis." Now with over 800 employees, Hologic's growth in China has been over twenty percent per year, an indication that Hong Kong is the perfect base for their operations.

PuraPharm www.purapharm.com

PuraPharm, a pioneering company dedicated to the internationalization and modernization of traditional Chinese medicine (TCM), was founded in 1998. With an investment of over HK\$200 million (USD\$26 million) in its state-of-the-art TCM research and production facilities in China's Guangxi province, PuraPharm is today a frontrunner in the industry. Their ISO-17025 (CNAS certified) in-house laboratory is regarded as one of the best TCM manufacturing facilities in Asia.

"It is very exciting to see how Chinese medicine is developing in China," says Mr. Abraham Yu-Ling Chan, PuraPharm's chairman. "Over the past decade, Hong Kong has not been leveraging its knowledge resources to modernize Chinese medicine. But in the next five years I believe we will see a big change, as TCM is becoming very popular here. Hong Kong is a very successful medical hub. It has credibility, attracts international scientists, and the work done here is of an international standard."

PuraPharm's main business supplies around 400 hospitals in China. The company produces concentrated TCM granules in sachets from prescriptions by professional practitioners. "It's not just modernizing TCM to add convenience," says Mr. Chan. "The granulation process allows us to standardize the treatments and test for heavy metals, making them safer." Crucially, PuraPharm is the only foreign-funded enterprise among six so-called Pilot Manufacturers of Concentrated Chinese Medicine Granules selected by China's SFDA. It has also been the only Chinese medicinal granule supplier of the Hospital Authority of Hong Kong for the past seven consecutive years.

Introducing TCM via PuraPharm's granules to a Western audience is next. "We have to show them that it's safe, that we have a quality control process," says Mr. Chan. "The next step is to demonstrate the effect of these products by doing clinical trials." With this in store, PuraPharm will no doubt continue to grow.



Abraham Yu-Ling Chan

Hong Kong Trade Development Council

www.hktdc.com

The non-profit Hong Kong Trade Development Council (HKTDC) was established in 1966 as the international marketing arm for Hong Kong-based traders, manufacturers, and service providers. With over 40 global offices, including 11 on the Chinese mainland, HKTDC's mission is to create and promote business opportunities for Hong Kong worldwide.



Ralph Shui-Sang Chow

"The major focus of our work is to organize the trade fairs in Hong Kong and to bring our Hong Kong companies to overseas markets," says Mr. Ralph Shui-Sang Chow, Director of Product Promotion. HKTDC attracts more than half a million buyers to

over 30 trade shows it holds annually. It features over 1.2 million registered buyers and 120,000 suppliers from Hong Kong, mainland China, and the rest of the world in its online marketplace, and publishes 15 product magazines with a readership of over five million. By organizing around 800 Hong Kong promotional events globally, reaching nearly 100,000 people each year, HKTDC also brings Hong Kong to the world. Six hundred international business missions visit Hong Kong a year with the assistance of HKTDC. Additionally, services such as HKTDC Business Matching help to connect companies who want to find the right partners.

Biotech is a key industry for Hong Kong and has been designated a "pillar of growth" by the government. HKTDC currently runs the annual Hong Kong International Medical Devices and Supplies Fair and receives biotech missions from myriad countries looking for collaboration. The organization also brings Hong Kong biotech companies to the rest of the world. Annually, it takes around 10 to 15 companies to the BIO International Convention, held in the United States. Last year, HKTDC also took biotech companies and organisations to Boston, calling on a medical school and the Massachusetts Institute of Technology to promote partnerships in biotech.

HKTDC's work related to biotech doesn't stop there. One anchor event for HKTDC is the Business of Intellectual Property Asia forum (BIP Asia), jointly organized by HKTDC and the Hong Kong Design Centre. "Biotech has the type of IP that needs a lot of legal protection and also financial services to facilitate its transaction," says Mr. Chow. "BIP Asia provides a platform to help all companies come together and facilitate the transaction."

Importantly for research and development in Hong Kong, HKTDC works closely with local R&D institutions to facilitate commercialisation of local innovation and technology by providing different outreach and marketing platforms. Mr. Chow explains: "We organize seminars and trade forums for them to publicize their latest achievements in the technology field, such as the ongoing series of Nanotechnology Forums co-organized with NAMI [the Nano and Advanced Materials Institute Limited] to promote their latest technology projects." To this end, Parker Robinson, the head of Corporate Communication at HKTDC, states that HKTDC also works closely with the Hong Kong Science and Technology Parks Corporation (HKSTPC). "Besides co-organizing promotional

"We have very close working relationships at many different levels in China...we believe there is tremendous potential for further collaboration between Chinese and overseas companies."

-Mr. Ralph Shui-Sang Chow

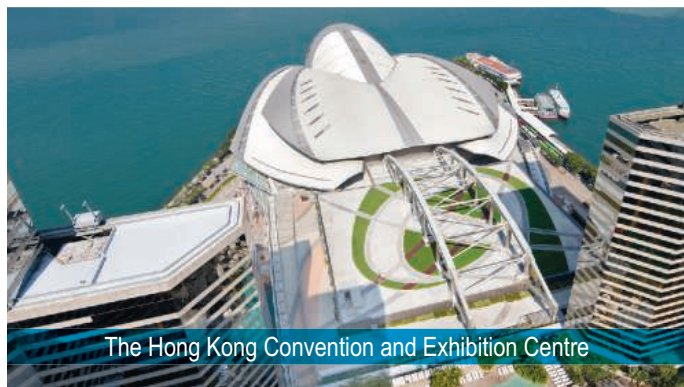
activities, we are keen to feature success stories of HKSTPC incubatees to highlight the capabilities and achievements of the local R&D sector," he says.

Relations with China are crucial for HKTDC, particularly with regard to biotech, an area of focus in China's 12th Five-Year Plan. "We

have very close working relationships at many different levels in China," says Mr. Chow. "We have 11 offices in mainland China where our colleagues maintain contacts in every province. We also bring Hong Kong delegations to visit Chinese cities and carry out many promotional activities throughout the country to champion various technology sectors."

Chinese delegations visiting Hong Kong are also an important part of facilitating business. To many Chinese companies, Hong Kong is their springboard to the global market. "Almost weekly, Chinese delegations come to Hong Kong," explains Mr. Chow. "We organize promotional activities and seminars to help them reach their potential buyers and clients, and arrange networking functions and business matching services to connect them to the world." Mr. Chow continues, "Hong Kong also provides sophisticated professional legal, financing, and accounting services that facilitate Chinese-overseas investment partnerships." A case in point was a Chinese delegation brought from Jiangsu province to Los Angeles last year.

"We believe there is tremendous potential for further collaboration between Chinese and overseas companies," concludes Mr. Chow. "And Hong Kong plays a very important role as a facilitator and also as a platform for these buyers and sellers to conclude their negotiations."



The Hong Kong Convention and Exhibition Centre

Invest Hong Kong (InvestHK), a department of the Hong Kong SAR Government, is responsible for helping overseas and mainland Chinese businesses to set up and expand in Hong Kong. These businesses are from a broad range of industries, including biotechnology, which has been identified by the Government as a growth industry under the “Innovation and Technology” pillar.

“Our goal is to attract companies that bring new expertise to Hong Kong, so biotech is an important sub-sector for us,” says Mr. Simon Galpin, director-general of Investment Promotion for InvestHK. “We have a lot of the world’s biggest companies already very well established here. But we’re keen to attract smaller, high-growth firms, including startups. Although these companies may only employ a few people at the outset, they need to use local service providers in Hong Kong. That in turn provides local job opportunities and has a spill-over effect.”

Founded in 2000, InvestHK strives to promote Hong Kong as a world-renowned center for business and a place for strategic investment. InvestHK provides information for companies thinking about entering Hong Kong and relocation advice for expatriates, including housing, schooling, and more. InvestHK also helps with business development, facilitates introductions to contacts and service providers, and provides marketing and public relations for a company’s launch and expansion. This year InvestHK will help over 300 companies set up and expand in Hong Kong.

One unbeatable advantage to conducting business in Asia’s premier business city is Hong Kong’s proximity to mainland China. Crucially, Mr. Galpin is keen to emphasize the meaning

of ‘one country, two systems’ in practice. “Why is Hong Kong unique?” he asks. “We explain those enduring advantages that haven’t changed since the 1997 handover: Rule of law, low and stable taxes, free movement of information, availability of capital, and people.”

“Second, we talk about the opportunities that arise or have arisen since Hong Kong reverted to Chinese rule,” adds Mr. Galpin. “On the business-to-business side, the fact is that we have hundreds of mainland companies coming here who want to use Hong Kong to go global. Hong Kong is a meeting point.”

A third advantage for biotech companies is that “we really are at the doorstep of the huge Chinese market. That is something that other competitors cannot challenge. They can’t move their countries closer to China!” quips Mr. Galpin. “We sit next to the world’s second biggest economy: if you are developing products that are ultimately going to be used in mainland China, it makes sense to be close to that market,” he says. Hong Kong’s location in the heart of Asia also makes countries like Japan, Korea, and all of South-East Asia easily accessible. Many of the continent’s key markets are less than four hours’ flight away and

half of the world’s population is located within five hours’ flight from Hong Kong.

A unique advantage for biotech in Hong Kong is its strong traditional Chinese medicine sector. “Hong Kong practitioners understand Chinese medicine very well and yet many of our practitioners have also studied Western medicine, so they are able to combine the Chinese and Western traditions together,” explains Mr. Galpin. “I am certain that this will result in many breakthroughs in the coming years.”

Beyond this, there are several other key advantages for companies setting up in Hong Kong. The city is a cosmopolitan one, able to attract talent worldwide to work and study. It has world-renowned universities that create top-level research and produce some of the best graduates globally. Businesses can draw also on talent from mainland China.

Mr. Galpin’s advice for companies thinking of locating to Hong Kong is, above all, to come and visit. “Come and see the facilities we have. Meet some of the companies already doing business and research here—it is a great way to understand the opportunities. And use our services as much as possible because they’re all free, customized, and confidential,” he says.

There are, of course, challenges, both in terms of attracting investment in biotech and encouraging small companies or startups to set up in Hong Kong. “People know Hong Kong as a leading financial center and there are many Fortune 500 companies here,” explains Mr.

Galpin. “But smaller companies sometimes assume that this is a market that isn’t for them. We see Hong Kong as the ideal starting point when foreign companies come into Asia, particularly those from North America and Europe. All you need is one Hong Kong dollar, one hour of your time, and one director. We’ll help a company as long as it commits to having just one person in Hong Kong.”



Simon Galpin

“We see Hong Kong as the **ideal** starting point when foreign **companies** come into Asia, particularly those from North America and Europe.”

—Mr. Simon Galpin



Hong Kong at night

The Hong Kong Applied Science and Technology Research Institute

www.astri.org

The Hong Kong Applied Science and Technology Research Institute (ASTRI) was set up in 2000 funded primarily by the government run Innovation and Technology Commission (ITC). Its three-fold tasks are to perform research and development for transfer to industry for commercialization, to develop technical human resources, and to bring industry and university R&D assets together. The organization's overarching goal is to help stimulate growth of technology-based industry in Hong Kong, including the health care sector. To this end, ASTRI focuses on five technology areas: communications technologies, consumer electronics, integrated circuit design, materials and packaging technologies, and biomedical electronics. It currently has a workforce of over 500 people, has close to 300 granted patents in China, the US, and other parts of the world, and has completed more than 300 cases of technology transfer in the form of technology licenses, research contracts, and more.



Francis Chee-Shuen Lee

ASTRI's Bio-Medical Electronics (BME) team was formed three years ago to expand information and communication technologies into biomedical applications required by the

Hong Kong community and industry. "BME works by interacting with health care and medical professionals and users to ask how R&D might help enhance their professional practices and applications," explains Dr. Francis Chee-Shuen Lee, vice president and R&D director of the Bio-Medical Electronics Team. "Interacting with industry takes place through individual contacts, forums, and conferences. BME also interacts with academic organizations to explore linking applications based on their upstream scientific or engineering outputs."

In the near term, BME's core focus is in telecare, digital pathology, and traditional Chinese medicine. One specific project under way is the development of instrumentation and methodology for amblyopia ("lazy eye") treatment. "In China, three to five percent [of the population] has this problem, mostly children," says Dr. Lee. In the past, eye-patches have been the only means of training the patient's weak eye (a method only effective for children under nine years old). "But our method provides specifically defined visual hardware and application software," says Dr. Lee. "It includes careful evaluation and analysis of the condition of the patient's weak eye. Data show improvements in patients 10 years and older, even adults, using the training." Currently, BME is in the process of working with an industrial partner in China to commercialize the technology.

The Nano and Advanced Materials Institute Limited

www.nami.org.hk

The Nano and Advanced Materials Institute Limited (NAMI) was established in 2006 with government and industrial funds and has an annual budget of around US\$20 million. The organization's staff of 110 is located at the Hong Kong University of Science and Technology (HKUST) and the Hong Kong Science Park, with laboratories in both locations.

NAMI, the only nanotechnology center in Hong Kong, conducts market-driven, demand-led development of nanotechnology and advanced materials. Its primary goals are to develop nanotechnology, to act as a focal point for market-driven R&D, and to train human resources to



Ka-Ming Ng

meet the future needs of both Hong Kong and the Pearl River Delta region in mainland China. NAMI focuses on five market sectors: sustainable energy, construction/building materials, environmental technologies, display and solid-state lighting, as well as biotechnology and health care products. In addition to in-house R&D, it funds projects and collaborates with researchers at universities in Hong

Kong and across the world.

NAMI has worked with academia to come up with important products, including oral capsules for the delivery of insulin and other biomolecules such as isoflavone, and engineering quality assurance solutions in the manufacture of Chinese herbal medicines.

"In biotech, one area that is important to us is Chinese herbal medicine," says Professor Ka-Ming Ng, CEO of NAMI and Chair Professor of Chemical and Biomolecular Engineering at HKUST. Another important project is a nanopreparation for the topical treatment of limb injuries. Local physicians have successfully used topical herbal pastes and patches to heal injured bones for hundreds of years. Research conducted by the Chinese University of Hong Kong, HKUST, and NAMI has identified key herbs that have healing effects through inflammation control, angiogenesis, and bone stimulation. A user-friendly patch developed to topically deliver the nanomized active ingredients has shown promising results.

"Products derived from biomaterials and related processing technologies ranging from biofuels to medical devices are expected to significantly impact our daily lives. NAMI is committed to contributing advances in this market sector," stresses Professor Ng.



City University of Hong Kong (CityU) is a dynamic higher education institution founded in 1984 that provides professional education and problem-driven research for the benefit of society. It has taken giant strides over the last few years and is now placed in the top 95 in the Quacquarelli Symonds (QS) World University Rankings 2012 and 12th in the QS Asian University Rankings 2012.



Way Kuo

Well-known for its engineering program, which was ranked 32nd in the Academic Ranking of World Universities published by Shanghai Jiao Tong University in 2012, today the University is ramping up its life science and biotech presence, using its strong record of academic achievement as leverage.

"Globalized higher education in the 21st century will be characterized by role differentiation," CityU President Professor Way Kuo explains. "Each university should look for niche areas in order to impact higher education. With life science and biotech taking root in Hong Kong, and to leverage our existing profile of neuroscience research, CityU is expanding into veterinary medicine and biomedical engineering."

Among the top talents recently recruited to CityU's new biomedical engineering program is Professor Ying Li (see p. 1649) who joined the department in 2009 from the University of Michigan in the U.S. Ten other faculty members have been recruited lately from such institutions as the Massachusetts Institute of Technology and Princeton University.

A key factor in CityU's expansion into life science and biotechnology, and its ability to attract both professors and students from across the world, is its international outlook.

"Each university should look for **niche areas** in order to impact higher **education**."

-Professor Way Kuo

Half of the faculty teaching the 20,000 students on campus are from overseas, and CityU aims to have 50 percent of its students spend at least one semester abroad at one of its many partner institutions, such as the University of California, Los Angeles, University of New South Wales in Australia, and Japan's Tohoku University.

In 2014, CityU hopes to launch a veterinary school in collaboration with Cornell University in the United States, with the objective of having the first American Veterinarian Medical Association-accredited veterinary program in Asia. The school will provide both undergraduate and postgraduate veterinary training, clinical training, and research into infectious diseases. It will address public health issues, the environment, and human-animal relationships.

With around 75 percent of human diseases—including severe acute respiratory syndrome (SARS) and avian influenza—originating in animals, Professor Kuo believes the new school will "add tremendous value to society."

Dr. Michael I. Kotlikoff, Dean of the College of Veterinary Medicine at Cornell, visited CityU in October for further discussions, agrees that food safety and food security are important issues in Hong Kong and mainland China.

Producing high-quality milk, for example, requires "not only equipment, investment, and cows, but also veterinarians," Dr. Kotlikoff says.

Crucially, Beijing's Tsinghua University will partner with CityU in developing the veterinary school's undergraduate program. The idea, Dr. Kotlikoff says, is to "train the trainers who will then go back and really impact the mainland."

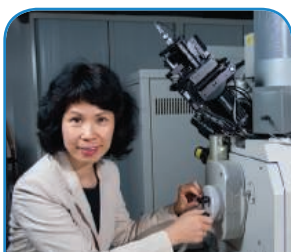
Meanwhile, CityU's international outlook represents a perfect bridge between the East and West for its many innovative faculty members.



Michael I. Kotlikoff

Professor Stella W. Pang

IEEE, AVS, and ESC Fellow, Chair Professor of Electronic Engineering, Department of Electronic Engineering



Stella W. Pang

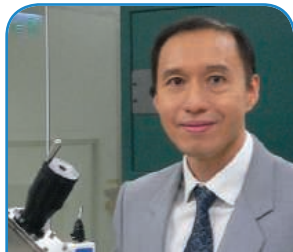
A Hong Kong native, Professor Pang arrived at CityU in 2011 after two decades in the Department of Electrical Engineering and Computer Science at the University of Michigan. This July, Professor Pang, who completed her Ph.D. at Princeton, formed an interdisciplinary research team to explore health diagnostics, monitoring, and treatment using

sensors and actuators. She hopes to build microsystems to target various diseases that, for example, could be related to hearing loss, eyesight loss, or Parkinson's disease. Research areas include nanofabrication technology, nanoimprinting, microfluidic systems for DNA analysis, nanostructures for cell growth, microelectromechanical systems (MEMS)-based chemical and biosensors, optical sensors for biomolecules, and micromachining technology and devices, among others.

CityU's strong reputation in engineering, combined with the university's focus on interdisciplinary work, is an advantage. "Being a young university allows us to explore different directions," she adds. "CityU is open to new ideas and wants to do something important and significant for society. I find that to be encouraging and attractive."

Professor Paul Kim-Ho Chu

APS, AVS, IEEE, and HKIE Fellow, Chair Professor of Materials Engineering, Department of Physics and Materials Science



Paul Kim-Ho Chu

Professor Chu relocated to a new 500 m² laboratory three years ago, focusing on materials and plasma surface engineering research in CityU's Department of Physics and Materials Science. It is the only lab of its kind in Hong Kong. His diverse research activities span plasma science and engineering, ion implantation, surface modification, functional thin films, biomaterials, semiconductor materials

and processing, optoelectronic materials, and nanotechnology.

Professor Chu's innovative applied research and industrial applications in plasma processing and instrumentation have resulted in one European, seven Chinese, and 12 U.S. patents. Current research activities include an improved spinal correction rod. "Usually nickel in the rod will diffuse, causing allergies in the patient," explains Professor Chu. "We keep the mechanical properties of the original rod, but prevent nickel from leaching out by adding a protective coating." Following successful clinical trials, he is currently in talks with an industrial sponsor about licensing.

Hong Kong's proximity to China is an advantage, says Professor Chu, who just received 6.5 million RMB (US\$1.04 million) in research grants from China to develop biomedical products over the next five years. In total, he has been granted over US\$14 million in research funding from agencies and companies. Professor Chu is ranked number one in Hong Kong for research output—producing around 100 papers per year—and is in the top 100 worldwide in materials science according to Essential Science Indicators, based on the number of citations.

Professor Shuk-Han Cheng

Department of Biology and Chemistry



Shuk-Han Cheng

"CityU's environment is highly conducive to interdisciplinary research," says Professor Cheng, who joined the university in 1997. "I work with engineers or physicists so that I can produce things that I could never create on my own." While some universities separate disciplines into different buildings, CityU has disciplines such as biology and engineering based in the same place. "This means you interact more with your colleagues and can build up collaborations by getting to know the person. It is much more solid in terms of interaction and much more fulfilling," says Professor Cheng.

This trend in interdisciplinary collaboration has also extended to student interactions. Three CityU students from mechanical engineering, marketing, and accounting backgrounds have recently won international business competitions based on a CityU student's doctoral research, and now boast multinational companies among their startup's clientele.

Professor Cheng has also branched out into regenerative

biology through her interdisciplinary work. Pioneering research includes looking into the role of Iroquois genes in vertebrate retinogenesis and cardiogenesis.

Professor Ying Li

M.D. (Beijing), FAGA (U.S.), Department of Biology and Chemistry

Chinese-born Professor Li was educated at Beijing Medical University before becoming a general surgeon and oral-maxillofacial surgeon at Nanjing Medical School in China. He joined CityU in 2009 after two decades at the Department of Internal Medicine at the University of Michigan as an associate research professor. He has received over US\$3.8 million in research grants from the National Institute of Neurological Disorders and Stroke in the U.S. His research interests include brain targets for chronic pain, central nervous system sensitization and plasticity, sensory signal transduction in the vagal primary afferent neurons, and vagus nerve stimulation therapy.

"CityU supports neuroscience very well," says Professor Li, who currently has laboratories on the CityU central campus and the southern city of Shenzhen in the Chinese mainland. "What we're doing is unique, as very few laboratories are investigating brain cortex synaptic plasticity and vagal afferent neural control of chronic pain using an in vivo model."

Professor Wen-Jung Li

Department of Mechanical and Biomedical Engineering

Professor Li completed his Ph.D. in aerospace engineering at the University of California, Los Angeles, and worked at The Chinese University of Hong Kong for more than a decade before joining CityU last year. His research interests range from MEMS sensors to nanobiotechnology and electrokinetic nano-assembly.

"MEMS/nano-based biotech research in Hong Kong is expanding," says Professor Li. "In the early 2000's, only a few research groups in Hong Kong were focused on using MEMS technology for developing motion-sensing technologies and microfluidic systems. In the past five years, based on the proposals funded by the Hong Kong Research Grants Council, many research groups in Hong Kong are now working on lab-on-a-chip technology—which combines MEMS and biomolecular detection technologies—for chemical or biological detection."

Professor Li's group also makes advances in micropower generators, microcell grippers, and carbon nanotube sensors. They have received worldwide recognition based on a number of citations and awards from flagship IEEE international conferences. Professor Li's current research using optically induced electrokinetics as a possible technology to biologically mark cancer cells has recently received a joint Croucher Foundation-Chinese Academy of Sciences grant.



Ying Li



Wen-Jung Li

The Hong Kong Polytechnic University

www.polyu.edu.hk

The Hong Kong Polytechnic University (PolyU) is a pioneer in application-orientated education and research within Hong Kong. Granted full university status in 1994, PolyU is today the territory's largest government-funded tertiary institution in terms of student headcount, serving the practical needs of both the local community and the wider world. Demonstrating innovation, as of mid-2011, PolyU had over 300 patents granted and 520 pending.



Chi-Ho To

PolyU is actively engaged in two biotech Areas of Excellence (AoEs), supported by the University Grants Committee (UGC) to spearhead development in research areas where Hong Kong competes internationally. It has been awarded funding for research in Chinese Medicine and Further Development as well as for the support of its Institute of Molecular Technology for Drug Discovery and Synthesis.

Areas of strength at PolyU include traditional Chinese medicine (TCM) modernization, food safety, myopia research, and biomedical ultrasound. One high-profile drug discovery project is the development of a new cancer drug that starves tumor cells through the depletion of arginine (a key nutrient for many cancer cells) and is showing great promise in clinical trials. On the food safety side, a demand for modern testing facilities led to the establishment of the Food Safety and Technology Research Center, hosted by the Department of Applied Biology and Chemical Technol-

ogy. According to the Food and Environmental Hygiene Department, in 2011 a total of 4,265 food complaints were handled—an increase of 14 percent over 2010. Researchers at PolyU are working hard to develop more sensitive and rapid food pathogen detection methods, assays, and devices.

Hong Kong has also grown into an excellent location for myopia research. "Here, patients are often very motivated and well-educated, so they are more willing to join clinical studies and try alternative methods to control myopia," explains Professor Chi-Ho To, associate head of the School of Optometry.

In an application-oriented higher education institute, which prides itself on research and innovation, collaboration and understanding are key. "The relationship between Hong Kong and China is growing," explains Professor Thomas Yun-Chung Leung from the Department of Applied Biology and Chemical Technology and Director of the Lo Ka Chung Centre for Natural Anti-Cancer Drug Development. "There are more and more collaborations. We help them to train students and postdoctoral fellows, while they provide the funding, power, and infrastructure."

Examples include the Hong Kong Scholars Program in which 50 top Chinese Ph.D. graduates work in Hong Kong (half of their salaries are financed by the mainland government), and the HK Ph.D. Fellowship Scheme, established by the Research Grants Council in 2009, which aims to attract the world's brightest students to study in Hong Kong and has many applications originating in China. PolyU is actively involved in both schemes.

“The global **population** is becoming progressively more myopic... It's no longer just a **national** or Asian problem.”

—Professor Chi-Ho To

Food Safety Technology

The Food Safety and Technology Research Center, hosted by the Department of Applied Biology and Chemical Technology, has won several awards for its work in improving food safety in Hong Kong. Dr. Ka-Hing Wong and Professor Samuel Chun-Lap Lo of the Department of Applied Biology and Chemical Technology, and Dr. Derek Siu-Wing

Or of the Department of Electrical Engineering have all won Gold Awards in the International Exhibition of Inventions of Geneva. Professor Lo and Dr. Or's collaborative invention is a portable and fast device for rapid identification of food-borne microorganisms. Dr. Wong was also awarded the Young Investigator Award of the 2011 International Conference on Food Factors.



Mo Yang

"Food Safety has been a major concern in Hong Kong, where most of the fresh food is exported from mainland China. We cannot control the source, so contamination is a big concern," explains Dr. Mo Yang, associate professor in the Interdisciplinary Division of Biomedical Engineering and a core member of the Food Safety and Technology Research Center, the first of its kind run by a higher education institution. The Center aims to raise the standards of food safety by providing research, consultancy, and training services to food industries in Hong Kong and the Pearl River Delta Region.

PolyU focuses on both developing devices and promoting food safety knowledge that can be used by the government, public, and the catering industry. This year, PolyU hosted the annual 2012 Functional Food Symposium (functional food, explains Professor Lo, refers to "food that



Samuel Chun-Lap Lo

has ingredients with specific functions that prevent diseases”).

Professor Kwok-Yin Wong, Chair of Chemical Technology and Dean of the Faculty of Applied Science and Textiles, researches fluorescent biosensors and electrochemistry. His work includes the use of environ-



Wing-Tak Wong

ment-sensitive dyes in the construction of protein-based biosensors. “Instead of using really expensive instruments to detect food contamination, we work to prepare agents that register unwanted contaminants by means of biosensors,” explains Professor Wong. “These are ideal for on-site food safety inspections at the front line of quality control. We can bring [the testing device] in a brief-

case. By contrast a full food safety lab is very expensive.”

For students of food safety, the advantages of studying at PolyU are plentiful. “We provide opportunities to place our students in the field,

actually on site,” points out Wing-Tak Wong, Director of Food Safety and Technology Research Centre, Department of Applied Biology and Chemical Technology. “Some of our arrangements are in China, particularly with government establishments. Students can help serve as ambassadors between China and Hong Kong, improving two-way communication.”

“Food safety testing and related research is now expanding in Hong Kong,” adds Dr. Yang. “There is a big demand for portable and rapid food safety screening devices.” Dr. Yang has developed a sensitive nanobiosensor device that can detect pathogen contamination at only 10 colony forming units per milliliter, within 30 minutes. He is also developing organic polymer/semiconductor-based field effect transistors (bio-FETs) for food pathogen detection. “The bio-FET is a promising approach for label-free, rapid, and direct detection of biological targets. It directly measures the conductance change when food pathogens bind to the detector surface,” he explains.

The Nature and Nurture of Myopia

PolyU’s Center for Myopia Research was established in 1998 as an area of strategic development that later became a Niche Area of Research, with total funding of HK\$34 million (US\$4.4 million).



Shea-Ping Yip

“The global population is becoming progressively more myopic, possibly because of the extensive amount of close work that we do. It’s no longer just a national or Asian problem—it is becoming a world problem,” explains Professor Chi-Ho To, associate head of the School of Optometry. “Although Myopia research is a PolyU niche area, we break boundaries; we involve microbiologists, geneticists, protein researchers, and physiologists,” he adds. “It is really a translational and interdisciplinary research area.”

Professor To’s team works on the biological and optical signals that regulate normal and myopic eye growth. They study the growth signals

in the eye during development “using proteomics approaches in which thousands of proteins can be visualized and compared,” says Professor To. “My lab has also demonstrated for the first time that the retina is capable of simultaneously integrating out-of-focus (defocus) images both in front of, and behind, the retina and guiding eye growth accordingly. This is an important finding in that it opens up a new opportunity to control myopia by manipulating optical inputs.” Professor To and colleagues have carried out a randomized clinical trial on myopia control using a novel contact lens called a defocus-incorporated soft contact (DISC) lens and have found that myopia progression can be retarded by 50 percent using this device. They are now optimizing the treatment by enhancing the dosage of anti-myopia optics.

Professor Shea-Ping Yip, associate head (Research) of the Department of Health Technology and Informatics, is looking at myopia from a genetics viewpoint. In Hong Kong, “the proportion of the population undergoing myopic degeneration, which can cause severe vision loss and even blindness, is going to be quite high,” says Professor Yip, who completed his Ph.D. in Human Genetics at the University College London.

He has collaborated with other international researchers in the Consortium of Refractive Error and Myopia (CREAM). “When I first started working in this area, there were only a few groups studying the genetics of myopia—now there are up to 40 groups in CREAM collecting

“Instead of using really expensive instruments to **detect** food contamination, we work to prepare agents that register unwanted **contaminants** using biosensors.”

—Professor Kwok-Yin Wong

data,” he says. “The trend is to look for genes and environmental factors that make us more likely to have myopia. We take a strongly multidisciplinary approach and join forces with scientists in other disciplines to solve common problems.”

Biomedical Ultrasound

“Ultrasound research in PolyU has been expanding dramatically, in the development of new technologies as well as in using ultrasound to study a variety of research questions,” says Professor Yong-Ping Zheng, acting head of the Interdisciplinary Division of Biomedical Engineering. “My team has made advances in a number of areas in biomedical and rehabilitative ultrasound, with a focus on the development of novel techniques for the assessment of soft tissues, including elasticity measurement, 3-D ultrasound imaging, and functional assessment of muscle, and their application through collaboration with researchers in different fields.”

Professor Zheng’s research and development in soft tissue characterization and elasticity measurement is internationally known, particularly for the team’s novel patented techniques of ultrasound indentation, water-jet indentation, and air-jet indentation.

One especially interesting new technique developed recently is the radiation-free assessment of scoliosis using 3-D ultrasound imaging, trademarked as Scolioscan. Previously, children with scoliosis could not be adequately screened or monitored for the progression and outcome of treatment as X-ray imaging created a radiation hazard. But with the new technique, developed based on PolyU’s pioneering research in musculoskeletal 3-D ultrasound imaging, they can be assessed frequently. A local company has licensed three related patents

from PolyU. Developed with a matching grant for commercialization purposes supported by the Government's Innovation and Technology Fund (ITF), a prototype is now ready for testing and clinical trials. The system not only generates an X-ray-like projected image for quick assessment, but also a 3-D virtual model of the spine in order to assess deformities in different visual planes.

The Scolioscan technology is important in Hong Kong—over three percent of children there are known to have scoliosis—and also globally. Professor Zheng suspects that more scoliosis sufferers will be diagnosed as more are screened using the new technique.

Another novel ultrasound technique for quantitative dynamic assessment of muscle function is called sonomyography and measures real-time changes in muscle architectural parameters. It can also be used

as a human-machine interface, for example for the control of a prosthesis. Professor Zheng coined the term in 2006 and has since been awarded a U.S. patent. "In addition to its application, this breakthrough has also triggered the need for the development of new processing algorithms for muscle ultrasound images, and new ultrasound probes and systems, leading to the development of a new

field. I believe that sonomyography is changing the field of muscle functional assessment," says Professor Zheng.

In addition to Professor Zheng's team, PolyU has others colleagues working on different aspects of biomedical ultrasound across a number of different departments.

Cancer Drugs

"Research in cancer therapy shows signs of expansion in Hong Kong... and at PolyU," say Professor Thomas Yun-Chung Leung and Associate Professor Thomas Wai-Hung Lo, both from the Department of Applied Biology and Chemical Technology and Lo Ka Chung Centre for Natural Anti-Cancer Drug Development. PolyU is researching anti-cancer

therapies, either protein drugs or natural products, together with Professor Larry Chow, associate head of the Department of Applied Biology and Chemical Technology. "We are using compounds derived from herbs, traditional Chinese medicines, and enzymes from humans or even bacteria. Most anti-cancer drugs are very toxic with a lot of side effects; we hope these compounds will be safer as well as more effective," explains Professor Leung.

Professor Leung and Dr. Lo developed pegylated recombinant human arginase (named BCT-100) in collaboration with Dr. Paul Ning-Man Cheng of Bio-Cancer Treatment International, Ltd. (BCT, a local biotech startup company, see p. 1643). The research was based on work suggesting that arginine depletion can starve tumor cells to death whilst leaving healthy cells relatively unscathed.

"We saw the potential of developing a cancer treatment that is free of many of the deleterious side effects commonly associated with cancer chemotherapy," explain Professor Leung and Dr. Lo. "The main constituent of this new drug is human arginase, an enzyme that degrades arginine." Modification of arginase by adding polyethylene glycol has been found to greatly prolong the enzyme's activity in the patient's blood.

"From the bench to the bedside: translational research is what we are doing here."

-Professor Thomas Yun-Chung Leung

Following highly optimistic preclinical results, BCT has put the new drug in phase 1 clinical trials in Hong Kong for liver cancer, with phase 2 currently ongoing. Recently, BCT-100 became Hong Kong's first ever drug to receive investigational new drug (IND) approval from the U.S. Food and Drug Administration (FDA) and thus clearing the way for clinical trials in the United States. This achievement is an important milestone in the development of the biotechnology and pharmaceutical industry in Hong Kong. Since then Professor Leung and Dr. Lo have developed a second generation anti-cancer drug molecule named BCA-PEG20, which has also shown promising results in preclinical tests.

"We believe that we have actually found a universal way of treating cancer. It's not only effective against liver cancer, but against many other types we have tested as well," says Professor Leung. "Cancer drug development is a global challenge," he adds, "and it takes on average around 14 years to get a drug to market. We believe that knowledge transfer is important for drug development—from the bench to the bedside: translational research is what we are doing here."



Yong-Ping Zheng



Thomas Yun-Chung Leung



Thomas Wai-Hung Lo

Hong Kong Baptist University

www.hkbu.edu.hk

Established in 1956, Hong Kong Baptist University (HKBU) has over half a century of experience educating Hong Kong's top students and is today ranked 111th worldwide by the 2010 Times Higher Education World University Rankings. Research is a top priority at HKBU: a University Grants Committee (UGC) Research Assessment Exercise, released in 2006, showed that three out of every four full-time HKBU academics were actively engaged in research. Particularly noteworthy is the School of Chinese Medicine (SCM), the first UGC-funded institution to provide full-time higher education in Chinese medicine in Hong Kong, and in which all staff members are involved in academic research. The school, founded in 1998 with a strong focus on education, has a clinical division and a total of 400 students (about 20 percent of whom are from mainland China).

HKBU's small size is an advantage says Professor Chris Kong-Chu Wong, head of the Department for Biology. "Less is more," he explains. "Our university is small, so teacher-student interaction is closer compared to other bigger universities. All final year students are actively involved in research, which is very different from other local universities. Different disciplines in the university also join together to share resources, another significant advantage."

Collaborations do not stop there. Hong Kong sits in a unique position, with roots in both the Chinese and Western worlds. This makes it the perfect place to blend Chinese and Western medicinal practice and research. Crucial for HKBU's prestigious SCM are collaborations

with other top universities across the world. Of particular importance is HKBU's close work with mainland China. "There is more space in China and better connections for the industry; manpower is cheaper and we can obtain grant money there," says Raymond Wai-Yeung Wong, HKBU's chair professor and associate head of the Department of Chemistry.

In February 2012, HKBU officially opened the Shenzhen Research Centre at the Shenzhen Virtual University Park in southern China with the aim of modernizing traditional Chinese medicine (TCM) and promoting research on materials science. Multidisciplinary research is at the new center's core, with researchers working together who hail from chemistry, biology, physics, and Chinese medicine backgrounds.

In March 2010, the Hong Kong Chinese Medicine Authentication Centre (HKCMAC) was opened, accompanied by the set up of A-Mark Quality Chinese Medicines Authentication Scheme—a mechanism

to ensure the safety and quality of Chinese medicine products through a series of stringent laboratory tests. Its aim, backed by the Hong Kong government, is to promote Chinese medicine products in

“There is more **space** in China and better connections for the industry; manpower is cheaper and we can obtain **grant money** there.”

—Professor Raymond Wai-Yeung Wong

the international market and boost consumer confidence in this age-old tradition under rigorous new testing. Overall HKBU's SCM, the largest in Hong Kong, is proving to be an invaluable research center and resource for biotech in Hong Kong.

The School of Chinese Medicine (SCM)

scm.hkbu.edu.hk

Professor Ai-Ping Lu

Dean of Chinese Medicine, SCM; Director of Institute for Advancing Translational Medicine in Bone & Joint Diseases



Ai-Ping Lu

"The SCM tries to bring Chinese medicine into the international arena in medical science," says Professor Lu (M.D., Ph.D.) who supervises both the SCM at HKBU and the Institute for Advancing Translational Medicine in Bone & Joint Diseases. "We are trying to build up the standards and protocols for Chinese medicine, including quality control and safety. We try to integrate medicines to produce

a combination of Chinese and Western medicine," adds Professor Lu, who is a leader in international translational medicine and received his Master's and Ph.D. degrees from the China Academy of Traditional

Chinese Medicine (now known as the China Academy of Chinese Medical Sciences).

As a former director of the Chinese Academy of Social Sciences, Professor Lu believes that there "should be cooperation between Hong Kong and mainland China." This is particularly crucial in traditional Chinese medicine. China not only provides funding and resources but plentiful patients for clinical trials in TCM. Professor Lu has worked with Peking University and the Kunshan government to establish the Academician Chen Xinzi Translational Medicine in Bone & Joint Diseases Studio in the Kunshan Small Nucleic Acid Biotechnology Research Institute, which will promote RNAi-based translational medicine research in bone and joint diseases, funded by one million RMB per year (US\$160,182). In particular, the studio will develop a state-of-the-art platform to modify herbal products using small nucleic acid biotechnology to produce smart therapeutic molecules.

Professor Lu has also completed clinical trials for classical anti-arthritis drugs combined with natural herbal products designed to combat

rheumatoid arthritis. Trials conducted in mainland China have provided evidence-based methods to establish an ideal therapeutic strategy with a traditional Chinese style for sub-typed rheumatoid arthritis. Lu has been invited by Guanghua Hospital in Shanghai to establish the Institute of Arthritis to collect human samples from rheumatic patients, forming the largest biobank of rheumatoid arthritis in the world. "I am looking for further close cooperation [with China] in the construction of a translational medicine hospital in the next five or 10 years," he says.

Professor Zhao-Xiang Bian

Associate Dean and Professor of the Clinical Division, SCM



Zhao-Xiang Bian

Professor Bian's research focuses on three areas: the standardisation of Chinese medicine, new drug development based on TCM theory, and integrating Chinese medicine and Western medicine. Key for Professor Bian is his work helping to establish CONSORT (Consolidated Standards Of Reporting Trials) for TCM, which promotes the development of reporting standards and of design quality

for randomized controlled trials utilizing TCM.

TCM drug development is an exciting frontier. Using a strategy of integrating clinical testing with systems biology approaches, research teams are currently screening active compounds or active fractions from Chinese medicinal plants in specific target areas involving inflammation, cancer, viral disease, and diabetes to determine if small molecule drugs can be developed. "A fast track for new drug development can be created encompassing classical formulations and modern drug development paradigms," says Professor Bian. His team argued in a 2011 review published in *Acta Pharmaceutica Sinica* that the annals of Chinese medicine, which has treated patients for thousands of years, can be used as a rich resource for discovering new drugs. Beyond this, they offer centuries of patient data that are not yet being utilized and can help to bring safe, effective new drugs to the market in a cheap and rapid fashion.

"We try to **integrate** medicines to produce a **combination** of Chinese and Western medicine."

-Professor Ai-Ping Lu

Integrative medicine is a new trend of our era. "Mounting evidence supports the idea that integrating classical wisdom from TCM with Western medicine can improve the effectiveness and/or safety

of current treatments for certain diseases, such as cancer and metabolic diseases. The case for the merits of classical wisdom continues to strengthen," says Professor Bian. One example is the Hemp Seed Pill (a classical Chinese herbal medicine) for functional constipation. A paper published last year in the *American Journal of Gastroenterology* by Professor Bian's team concludes that this treatment is both safe and effective for alleviating excessive constipation. "Hong Kong is a very good place for integrative medicine development," Professor Bian concludes. "International teams can develop Chinese medicine here to help create new medicines."

"Worldwide, more and more people are interested in **Chinese** medicine. The use of **herbal** medicine is becoming more popular."

-Professor Zhong-Zhen Zhao

Professor Zhong-Zhen Zhao

Associate Dean and Professor of the Teaching Division

Professor Zhao is an internationally renowned scholar of Chinese medicine. For 30 years, Professor Zhao has focused his research on pharmacognosy and the authentication of Chinese medicine. In 2010, his "Encyclopedia of Medicinal Plants" was awarded a national prize of outstanding publishing, the highest government award in the news and publication field.



Zhong-Zhen Zhao

"Worldwide, more and more people are interested in Chinese medicine. The use of herbal medicine is becoming more popular," explains Professor Zhao. "At the same time, however, the quality of the herbs is also raising international concern. How we ensure the safety of medications is very important." In view of this, HKBU has established a standard authentication procedure which has been recognised by the international community.

Key is the creation of a database of herbal medicines (including a specimen center of authenticated herbal medicine and a museum of Chinese medicine) and a quality control platform for herbal medicines which have both been founded at the SCM in HKBU. Routine and innovative morphological authentication techniques have been used to authenticate herbal medicines.

"Hong Kong is a bridge to mainland China and to the rest of the world," says Professor Zhao. "It is also the window to show Chinese medicine to international society."



The Faculty of Science

Professor Ricky Ngok-Shun Wong

Acting Associate Vice-President and
Associate Dean of the Faculty of Science
biol.hkbu.edu.hk/?page_id=802



Ricky Ngok-Shun Wong

Originally a biochemist, Professor Wong believes that “interdisciplinary research is very important right now.” Professor Wong received his Ph.D. from the University of Oklahoma Health Sciences Center in Oklahoma City, U.S. and has experience in the biotechnology industry. He is responsible for setting up the biotechnology concentration of the applied biology program at HKBU.

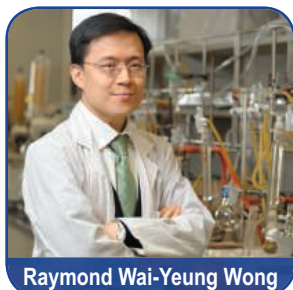
In 1998, Professor Wong started his current research on the molecular pharmacology of ginseng, particularly the pharmacologically active ingredients, ginsenosides, and their effect on microRNA expression and biogenesis leading to angiomodulation.

“We discovered that the two types of ginsenosides: protopanaxatriols (PPTs) and protopanaxadiols (PPDs), possess opposing effects on angiogenesis,” he explains, proving that ginseng is an adaptogen. Since this discovery, Professor Wong has addressed different ginseng-mediated activities in various models including angiogenesis, tumors, wound healing, anti-aging, adipogenesis, diabetes, and influenza.

A paper co-authored by Professor Wong in *Chinese Medicine*, entitled “Pharmacogenomics and the Yin/Yang actions of ginseng: anti-tumor, angiomodulating and steroid-like activities of ginsenosides” is today still the most accessed article for the journal with over 30,000 downloads since online publication in 2007.

Professor Raymond Wai-Yeung Wong

Chair Professor and Associate Head, Department of Chemistry
chem.hkbu.edu.hk/rwywong



Raymond Wai-Yeung Wong

Trained as a chemist, Professor Wong previously worked in traditional synthetic chemistry. Today, Professor Wong looks into basic scientific research and applications of new molecular functional materials with photofunctional and bio-imaging capabilities. He has a keen interest in investigating the potential applications of novel organic and metal-organic compounds in treating both solid and

non-solid tumors as well as hematological diseases such as platelet disorders and thalassaemia. He is also interested in drug discovery and drug delivery, especially bifunctional molecules able to both target a malignant cell and kill it. Of importance are his collaborations with the Research Development Division from the School of Chinese Medicine at HKBU to make use of *Phyllanthus urinaria* extract as an antidote for hepatotoxicity after overdose administration of Panadol (acetaminophen). The collaborative team has shown that *P. urinaria* extract works well in animal models and is now working with a Hong Kong biotech-



Phyllanthus urinaria

nology company to conduct clinical trials in the medical school of a local university. “Such work is expected to provide promising lead compounds for new drug development, and in vivo tumor profiling and diagnosis using two-photon bio-imaging techniques,” says Professor Wong.

Professor Chris Kong-Chu Wong

Director, Croucher Institute for Environmental Sciences
Head, Department of Biology
biol.hkbu.edu.hk/?page_id=426

Professor Chris Kong-Chu Wong specializes in environmental research. His current research interests include osmosensing mechanisms and osmoregulatory functions of fish gill cells, functional characterization of human glycoprotein hormones STC1 and STC2 in carcinogenesis, and environmental contamination and mechanistic actions of emerging chemical pollutants.

“We do environmental diagnostics,” says Professor Wong, who has been working in the areas of endocrinology and toxicology for over a decade. He now focuses on how pollutants affect human growth and health.

“Ten years ago, we collected different kinds of environmental samples for the measurement of different environmental pollutants. We are now moving to the measurement of pollutants in human body fluids such as blood and urine samples.” With respect to human disease, Wong’s laboratory is focused on human infertility, metabolic disorders, and the correlation between blood levels of pollutants and the occurrence of systemic lupus erythematosus, an autoimmune disease. This shift to researching the effects of environmental pollution is new to Hong Kong. China poses its own pollution problems: high levels of mercury are found in both the effluent from coal-burning power plants and in cheap creams used to whiten the skin. “Following patients from exposure to disease development has a very long time lag,” says Professor Wong. “We look for hidden susceptibilities that may be triggered, leading to metabolic disease.” Professor Wong’s research is aimed at understanding and combating the development of these disorders.



Chris Kong-Chu Wong

Academic Excellence

University of Hong Kong

www.hku.hk

The University of Hong Kong (HKU), located on Hong Kong island, was established in 1911 and is the city's oldest university. HKU sits at the top of the Hong Kong league tables, ranked 23rd globally according to the Quacquarelli Symonds World University Rankings 2012. HKU has the most Academicians of the Chinese Academy of Sciences of any local institution, and many of its scholars are amongst the top one percent in their field according to ISI's Essential Science Indicators.

Importantly for biotech, HKU's Clinical Trials Centre at the Li Ka Shing Faculty of Medicine was established in 1998 with the mission of enhancing global health care by attracting and facilitating clinical research on new drugs, medical devices, and other medical products, methods, and procedures, whilst ensuring compliance in terms of subject protection, scientific validity, and data integrity. HKU's Centre for Genomic Sciences, also part of the Li Ka Shing Faculty of Medicine, offers genomics, proteomics, and bioinformatics services. The center was established to facilitate the translation of knowledge into applications and is at the forefront of genomics research.

HKU's Technology Transfer Office helps those with inventions and patent applications to find funding and turn discoveries into business opportunities through licensing and commercialization.

Chinese University of Hong Kong

www.cuhk.edu.hk

The Chinese University of Hong Kong (CUHK) is located in the New Territories near the Hong Kong Science Park. Founded in 1963, CUHK



Hsiang-Fu Kung

is ranked 15th in Asia according to the Times Higher Education World University Rankings 2011–2012. It is renowned for its research in the sciences and has a top medical school, clinical trial center, Knowledge Transfer Office, and the affiliated Hong Kong Institute of Biotechnology offering downstream support to the industry. The University houses four state key laboratories in collaboration with

the Ministry of Science and Technology of China that produce research of national importance.

Professor Hsiang-Fu Kung, research professor of Virology at the Stanley Ho Center for Emerging Infectious Diseases and the School of Biomedical Sciences at CUHK, specializes in molecular genetics, molecular oncology, and virology. He looks at diseases such as human immunodeficiency virus, hepatitis B virus, and seasonal flu, and his diverse research interests include bacterial genetics and metabolism, enzymology, gene regulation, cytokines, and oncogenes. Infectious diseases are "always a major health problem in Hong Kong because of our special geographic location, the society, and the weather," explains Professor Kung.

For Professor Kung, CUHK's proximity to mainland China is a huge advantage, as it has a multitude of patients with infectious diseases, says Professor Kung, a member of the Chinese Academy of Sciences and an honorary professor at Peking Union Medical College. "My laboratory has enjoyed excellent collaborations with scientists in mainland China. We have established a wonderful friendship and collaborated very successfully on many international projects," he adds.

Hong Kong University of Science and Technology

www.ust.hk

The Hong Kong University of Science and Technology (HKUST) was founded in 1991 and is currently ranked 62nd worldwide and 7th in Asia (Times Higher Education World University Rankings 2011–2012). HKUST's focus on Biological Sciences and Biotechnology, considered one of five high-impact areas, is exemplified by the marine science research of Professor Pei-Yuan Qian, founding director of the university's state-of-the-art marine laboratory.

Professor Qian, who is in the Division of Life Sciences, researches marine invertebrates. His research centers on the isolation and identification of non-toxic antifouling compounds and drug leads from marine organisms, the interaction between settling larvae and biofilm dynamics, larval 'omics, and microbial metagenomics.

"We are pioneers in several areas of larval biology research, such as larval 'omics," explains Professor Qian. "We are one of the world's leading labs in both the larval biology and biofouling/antifouling research, and the Environmental Science Program at HKUST is currently ranked 32nd in the world. Hong Kong itself has become a center of larval biology research."

Professor Qian directs a collaborative research project under the Global Collaborative Research Program of King Abdullah University of Science and Technology (KAUST) to study microbial metagenomics and bioactive compounds from the Red Sea. "We have many collaborations in mainland China," says Professor Qian. "If we were located in North America or Europe this wouldn't be possible. In Hong Kong we also enjoy academic freedom and an independent faculty."



Professor Pei-Yuan Qian (left) showing guests around his marine laboratory

Biotech Successes in Hong Kong

The biotech industry in Hong Kong has leveraged the city's unique advantages: proximity to the Chinese mainland providing opportunities for research and development and a vast potential market, plus a supportive business environment that attracts international talent and has

a respected IP and common law system. Additionally, the Hong Kong Science Park (HKSP) provides a platform for both startup and established biotech firms to develop R&D and commercialization of products (see p. 1642). Below is a selection of successful local companies.

Angenomics, Ltd. is a leader in veterinary diagnosis, providing services and detection kits for veterinary pathogens including Avian Influenza H5 and H7, Foot-and-Mouth Disease, Classical Swine Fever, and Newcastle Disease, among others. The Avian Influenza diagnostic kits were the first imported RNA-based diagnostic kit to be registered with the Japan Ministry of Agriculture, Forestry and Fisheries.

www.angenomics.com

BioRx, Ltd. is a biotechnology startup company and a member of HKSP's Incu-Bio program located on site at the park. It develops "high-quality, low-cost biological products (cytokines, antibodies, vaccines) for cancer/immunology research use in pharmaceutical industry R&D departments, university and research institute laboratories, and hospitals," explains BioRx's founder Professor Xie Yung.

The Food Safety Laboratories, Ltd. (TFSL) is a pioneer in molecular food testing, with laboratories in both Hong Kong and Beijing. TFSL also offers molecular test kits for GMO testing, authentication, and pathogen detection across three amplification assay platforms. www.foodsafetylabs.com

New-A Innovation aims to advance health care by providing life-saving oxygen therapeutic products. One of its products used for treating canine anemia and hemorrhagic shock, Oxapex™, has received ACVM registration in New Zealand as a veterinary medicine. Currently, the applications for marketing authorization to European Medicines Agency and U.S. Food and Drug Administration are under way. New-A Innovation owns a certified GMP manufacturing facility in Hong Kong and facilities in New Zealand and China.

www.newainnovation.com

Hai Kang Life Corporation, Ltd.

Founded in 1999, Hai Kang Life Corporation, Ltd. (HKLife) is a pioneering molecular diagnostics company. Situated in HKSP, HKLife develops biotech applications and systems with the mission to revolutionize the practice of clinical diagnostics, providing effective platforms for point-of-care applications focused on personalized medicine and pre-emptive surveillance of emerging pathogens and diseases.

HKLife develops new products utilizing DNA-based technologies including the novel *EFADchip*® (Electric Field Assisted Diagnostic) technology. The system incorporates patented electric-field tech-

nology which will allow for the detection of multiple pathogens in a simple and cost-effective manner. The system's simplicity and user-friendliness allows for effective diagnosis in rural areas, with the initial product releases intended for blood screening, fever-causing pathogens, and respiratory disease diagnosis. In the future, this technology could assume a significant role in personalized medicine, disease surveillance, prognosis, theranostics, and even cancer detection. The *EFADchip*® is poised to deliver to the world innovative and cutting edge clinical solutions for the future.

www.haikanglife.com

CK Life Sciences Int'l (Holdings), Inc., a member of the Cheung Kong Group, is listed on the Hong Kong Stock Exchange. Pursuing the mission of improving quality of life, CK Life Sciences is engaged in the business of research, development, commercialization, marketing, and sale of health and agriculture-related products in the areas of human health and environmental sustainability. A number of the company's inventions have been granted patents in the United States. www.ck-lifesciences.com

DNA-TECH, Ltd. aims to integrate DNA-based products and services into daily life. Established in 2000, DNA-TECH provides testing kits and fingerprinting services which include prenatal testing, parentage testing, identification of relatives, forensics, and DNA profiling.

www.dnatech.com.hk

PolyGum Technologies, Ltd., an incubatee in HKSP, is focusing on material science R&D. "Our first government-funded product utilizes a proprietary and patented formulation approach to improve the compliance and versatility of the topical drug delivery system," said Dr. Bernard Pak-Li Chan. "We strive to make existing materials smarter so as to improve people's health and quality of life."

www.polygum-technologies.net

The Santai Eco Fishery, Ltd. fish farming system and technologies are based on All Weather Eco Fishery Technology (AEFS), a commercially proven technology. The state-of-the-art system utilizes a specially designed "treatment stack" that assures the most complete water treatment. All water on the farms is recycled, lowering energy consumption, while guaranteeing removal of harmful chemicals, antibiotics, pesticides, and herbicides. www.santaieco.com/en/

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Science Careers

From the journal *Science*



"Chang Jiang Scholars" and other faculty positions available in Nankai University

Nankai University locates in Tianjin City, the third largest city in China and 30 min away from Beijing by a high-speed train. Nankai University is one of the key national universities directly under the jurisdiction of the Ministry of Education of China, and also a "211 Project" and "985 Project" university in China. Nankai University is the center for both education and academic research, and has materialized a series excellent achievements, with the quality and the quantity of the research papers, projects, funds, and prizes are among the forefront of the national universities of China. Premier Enlai Zhou, the world-wide known mathematician Shiing-shen Chern, physicist Ta-you Wu and playwright Yu Cao are alumni of Nankai University. Nankai University is recruiting for outstanding investigators to occupy the following honorable positions. These positions will not be closed for next several years.

1. Permanent and visiting professors of "Chang Jiang Scholars Program": In addition to the requirements defined by the program (please see: <http://www.changjiang.edu.cn/>), the successful applicant with good healthy conditions should be an internationally known investigator with well-known achievement in the field, a strong leadership in guiding a first-class research team and a high capability in management and organization.

2. Professors and Associate Professors of "The National Thousand (Young) Talents Program", "The State (Young) Special Support Plan", and other high-level talent programs: In addition to the requirement defined by the program, such as "The National Thousand Talent Program" (<http://www.1000plan.org/>), the applicant with good healthy conditions should be a well-established and highly innovative scientist with strong academic records and leadership. The applicant for a young program should be able to demonstrate the potency to be an outstanding scientist in the future with the support from Nankai University.

Salary, start-up package and benefits: According to the policy recently issued by Nankai University, such as the "The Program Supporting Hundred High-level Talents", "The Plan Cultivating Hundred Young Academic Leaders", the recruited faculty at different academic levels will be supported with competitive salary, the start-up package (competitive start-up funds, newly renovated office/lab and experienced assistants), housing allowance, medical insurance and other possible benefits. All of the above offers are negotiable.

Contact us: The interesting candidates should send curriculum vitae in both of English and Chinese, the first page of 5 publications, statement of research interests/plans and at least three references to: Mr. Feng Li and/or Mr. Yuechao Wang, The Department of Human Resources, Nankai University, 94 Weijin Road, Tianjin, China, 300071; Tel: 0086-22-2350-8595; Fax: 0086-22-2350-1586; Email: nkuniversity@nankai.edu.cn. You will be contacted once we receive and finish the evaluation of your application for the position.

Nankai University is an Equal Opportunity Employer.

TENURE TRACK FACULTY POSITION IN CANCER RESEARCH

Case Western Reserve University School of Medicine Case Comprehensive Cancer Center

The Case Comprehensive Cancer Center (<http://cancer.cwru.edu/>), a National Cancer Institute-designated Comprehensive Cancer Center at CWRU, with affiliates at University Hospitals Case Medical Center and Cleveland Clinic, invites applications for tenure track faculty positions at the level of Assistant and Associate Professor in cancer biology. Candidates should have a doctorate and post-doctoral research experience. Candidates at the Assistant Professor level should provide a record of scholarly activity and external funding and have the potential to advance in cancer research. Candidates at the Associate Professor level should have a nationally-funded program and an outstanding record of cancer research achievements. Target areas of interest should be aligned with one of the Cancer Center's scientific programs, including regulation of cell proliferation and apoptosis, signal transduction, cell cycle regulation, DNA damage and repair, chromatin and epigenetics, cancer genetics, cancer stem cells, breast cancer, ovarian cancer, or colon cancer. Individuals with expertise in high through-put genomic methods are particularly encouraged to apply. Priorities include innovative discovery research coupled with an interest in translational clinical disease-oriented cancer research.

The successful candidate will have a primary appointment in the Cancer Center or a basic science department at the medical school such as Biochemistry (<http://www.case.edu/med/biochemistry/>), Molecular Biology & Microbiology (<http://www.case.edu/med/microbio/>), or Pharmacology (<http://pharmacology.case.edu/>).

Please send curriculum vitae, a list of three or more references, and a cover letter outlining your research interests electronically to: cancersearch@case.edu. Please include "Cancer Research Faculty Search" in the subject line.

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. Women, veterans, members of underrepresented minority groups, and individuals with disabilities are encouraged to apply.

Case Western Reserve University provides reasonable accommodations to applicants with disabilities. Applicants requiring a reasonable accommodation for any part of the application and hiring process should contact the Office of Inclusion, Diversity and Equal Opportunity at 216-368-8877 to request a reasonable accommodation. Determinations as to granting reasonable accommodations for any applicant will be made on a case-by-case basis.



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STRITCH SCHOOL of MEDICINE

DEPARTMENT CHAIR

The Loyola University Chicago Stritch School of Medicine has started a national search for the next Chair of the Department of Molecular Pharmacology and Therapeutics. The department engages in multidisciplinary research in neuro-, cancer, and cardiovascular pharmacology, with particular strengths in cellular signal transduction. The Department offers graduate training in programs leading to Ph.D., M.D./Ph.D., M.S., and M.S./M.B.A. degrees with a pharmacology emphasis. The department seeks an individual with strong leadership skills and the vision to develop and maintain robust, collaborative basic and translational research programs, and continue the strong commitment to graduate and medical education. The successful applicant will have a Ph.D. and/or M.D. degree, a strong record of academic achievement, a significant level of past and current extramural research funding, extensive experience in graduate and medical student education and/or success in drug discovery and translational research in the pharmaceutical industry.

Interested applicants should submit a cover letter, a description of their leadership vision, curriculum vitae and the names of five references by e-mail to **Michael Nishimura, Ph.D., Chair of the Search Committee (Pharmacology-Search@luc.edu)**. Review of applications will begin **January 1, 2013** and all applications and nominations will be considered until the position is filled or until an adequate applicant pool is established.

The Loyola University Chicago Stritch School of Medicine is a Catholic Jesuit institution dedicated to excellence in education, research, service and health care and is an Affirmative Action, Equal Opportunity Employer, and encourages applications from women, minorities, and others who can contribute to the University's research, teaching, and service missions.

Additional information about the Department and the Chair search can be found at: <http://www.stritch.luc.edu/depts/pharmacology/index.cfm>



Tenure-Track Assistant Professor Position in Genetics Department of Biological Sciences

The Department of Biological Sciences at Oakland University invites applications for a tenure-track Assistant Professor position to be filled by August 2013. We seek candidates whose research plans are focused on key questions of modern genetics and genomics. A Ph.D. and at least two years post-doctoral experience are required, as well as a strong research track record evidenced by publications. Lab space and competitive start up package will be provided. The successful candidate is expected to develop a vigorous, extramurally funded research program, to teach genetics effectively at the undergraduate and graduate levels, and to mentor graduate students (MS and Ph.D.).

The Department of Biological Sciences (<http://www2.oakland.edu/biology/>) is a vibrant and growing research-oriented department with active graduate programs at the Master's and Ph.D. level. The department has faculty in a broad range of disciplines, and within the field of genetics faculty interests include functional genomics, developmental genetics, human genetics, and evolutionary biology. Oakland University is a state-supported institution of 20,000 students. It is located on a beautiful 1,600-acre campus in the heart of Oakland County, 25 miles north of Detroit.

Applications should be submitted by January 28, 2013. Applications are to be submitted online at <http://academicjobs.oakland.edu/postings/575> and should include a cover letter, curriculum vitae, detailed statement of research plans, a statement of teaching interests, three representative publications, and a list of three or more references. Questions should be addressed to **Doug Wendell, Genetics Search Chair, Department of Biological Sciences (wendell@oakland.edu)**.

Oakland University is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.



CHAIRPERSON DEPARTMENT OF VETERINARY MICROBIOLOGY AND PATHOLOGY WASHINGTON STATE UNIVERSITY PULLMAN, WA

The College of Veterinary Medicine at Washington State University seeks a distinguished researcher and academic leader to be Chairperson of the Department of Veterinary Microbiology and Pathology (VMP) (<http://www.vetmed.wsu.edu/depts-vmp/index.aspx>). As one of the foremost pathology departments in the nation, VMP's mission is to enhance animal and public health through training veterinary medical students, graduate students and residents, conducting global outreach, and performing biomedical research in immunology, infectious diseases, food safety, genomics, and pathology. VMP's strong, extramurally funded research programs benefit from close collaborative ties with the USDA/ARS Animal Disease Research Unit, and other College of Veterinary Medicine entities including the Washington Animal Disease Diagnostic Laboratory, the newly founded Paul G. Allen School for Global Animal Health, and the School for Molecular Biosciences. VMP excellence in teaching is supported by the first-in-the-nation College Teaching Academy, whose mission is to enhance educational enterprises at all levels. The Chairperson's portfolio includes fiscal management of VMP, participation in and promotion of mentoring and professional development for faculty members, and fostering curricular development and scholarship in teaching. The successful candidate will have a record of academic leadership, productive, sustained, extramurally funded, internationally recognized research, and excellence in graduate education. Applicants must hold an earned doctorate in microbiology, immunology, pathology, veterinary medicine, medicine or a related discipline and be eligible for academic appointment at the level of Professor.

Review of applications will begin **January 7, 2013**. To apply or obtain more detailed information go to www.wsujobs.com. For questions or confidential expressions of interest about this position candidates may email or telephone **Ms. Sue Zumwalt** at szumwalt@vetmed.wsu.edu or 509.335.6027.

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Max Planck Institute for the Physics of Complex Systems

Center for Systems Biology at Dresden

Max Planck Institute of Molecular Cell Biology and Genetics



Max Planck Institute
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The Center for Systems Biology at Dresden (CSBD) in Germany announces the opening of positions in the

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The Center provides a vibrant and collaborative research environment with a strong commitment to the interdisciplinary training and career development of postdoctoral fellows. Successful candidates will benefit from close collaborations with scientists from the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), the Max Planck Institute of the Physics of Complex Systems (MPI-PS) and the Technical University Dresden.

ELBE Postdocs are awarded on a competitive basis through the post-doctoral program of the Center. To foster collaborations, fellows will usually be affiliated with two Principal Investigators working in different disciplines. For details about the application procedure, please visit our website <http://www.mpg-sysbio.de/jobs.html>.

The Max Planck Society is an equal opportunity employer: handicapped individuals are strongly encouraged to apply. The Center for Systems Biology, the MPI-CBG and the MPI-PS aim to increase the number of women in scientific positions. Female candidates are therefore particularly welcome.



MAX-PLANCK-GESELLSCHAFT



Cold Spring Harbor Laboratory 2013 Meetings & Courses

Meetings

Meeting dates / abstracts due

From Base Pair to Body Plan – Celebrating 60 Years of DNA

February 28 - March 3 / December 14, 2012

Single Cell Analyses

March 6 - March 9 / January 4

Systems Biology: Networks

March 13 - March 16 / January 11

Computational Cell Biology: The Interplay between Models & Experimentation

March 19 - March 22 / January 11

RNA & Oligonucleotide Therapeutics

April 10 - April 13 / January 25

Synapses: From Molecules to Circuits & Behavior

April 16 - April 20 / February 1

Cancer Biology & Therapeutics

April 23 - April 27 / February 8

Telomeres & Telomerase

April 30 - May 4 / February 15

The Biology of Genomes

May 7 - May 11 / February 22

The Ubiquitin Family

May 14 - May 18 / March 1

Retroviruses

May 20 - May 25 / March 8

78th Symposium: Immunity & Tolerance

May 29 - June 3 / March 15

Wiring the Brain

July 18 - July 22 / May 3

Metabolic Signaling & Disease: From Cell to Organism

August 13 - August 17 / May 31

Eukaryotic mRNA Processing

August 20 - August 24 / June 7

Mechanisms of Eukaryotic Transcription

August 27 - August 31 / June 14

Behavior & Neurogenetics of Nonhuman Primates

September 6 - September 9 / June 21

Eukaryotic DNA Replication & Genome Maintenance

September 9 - September 13 / June 28

Microbial Pathogenesis & Host Response

September 17 - September 21 / July 8

Stem Cell Biology

September 24 - September 28 / July 12

Neurobiology of Drosophila

October 1 - October 5 / July 19

Cell Death

October 8 - October 12 / July 26

Genome Informatics

October 30 - November 2 / August 16

Cell Biology of Yeasts

November 5 - November 9 / August 23

Precision Medicine: Personal Genomes & Pharmacogenomics

November 13 - November 16 / August 30

Harnessing Immunity to Prevent & Treat Disease

November 20 - November 23 / September 6

Plant Genomes & Biotechnology: From Genes to Networks

December 4 - December 7 / September 20

Rat Genomics & Models

December 11 - December 14 / September 27

Courses

Course dates / applications due

Workshop on Leadership in Bioscience

February 22 - February 25 / January 11

Protein Purification & Characterization

April 3 - April 16 / January 31

Quantitative Imaging: From Cells to Molecules

April 3 - April 16 / January 31

Cell & Developmental Biology of Xenopus

April 5 - April 16 / January 31

Workshop on Autism Spectrum Disorders

June 5 - June 11 / March 15

Single Cell Analysis

June 5 - June 18 / March 15

Advanced Bacterial Genetics

June 5 - June 25 / March 15

Ion Channels & Synaptic Transmission

June 5 - June 25 / March 15

Mouse Development, Stem Cells & Cancer

June 5 - June 25 / March 15

Vision: A Platform for Linking Circuits, Perception & Behavior

June 12 - June 25 / March 15

Statistical Methods for Functional Genomics

June 21 - July 3 / March 15

Workshop on Pancreatic Cancer

June 26 - July 2 / March 15

Drosophila Neurobiology: Genes, Circuits & Behavior

June 28 - July 16 / March 15

Frontiers & Techniques in Plant Science

June 28 - July 18 / March 15

Advanced Techniques in Molecular Neuroscience

July 2 - July 18 / March 15

Proteomics

July 9 - July 24 / March 15

Biology & Disorders of Learning & Memory

July 20 - August 2 / March 15

Computational Cell Biology

July 23 - August 12 / March 15

Eukaryotic Gene Expression

July 23 - August 12 / March 15

Yeast Genetics & Genomics

July 23 - August 12 / March 15

Imaging Structure & Function in the Nervous System

July 24 - August 13 / March 15

Synthetic Biology

July 30 - August 12 / March 15

Cellular Biology of Addiction

August 6 - August 12 / April 15

Programming for Biology

October 14 - October 29 / July 15

X-Ray Methods in Structural Biology

October 14 - October 29 / June 15

Computational & Comparative Genomics

November 6 - November 12 / July 15

Antibody Engineering & Phage Display

November 6 - November 19 / July 15

Advanced Sequencing Technologies & Applications

November 12 - November 24 / July 15

The Genome Access Course

April 21-23, July 18-20, November 17-19

Cold Spring Harbor Laboratory Meetings & Courses Program

1 Bungtown Road
Cold Spring Harbor, New York 11724
Phone: 516-367-8346
www.cshl.edu/meetings
meetings@cschl.edu



CSHL course on an evening sail.



ASSISTANT PROFESSOR OF PHARMACOLOGY/TOXICOLOGY

Applications are invited for a tenure-track position at the level of Assistant Professor in Pharmacology and Toxicology at the University of Kansas, School of Pharmacy. The Pharmacy School at the University of Kansas has a strong history of competitive research and ranks second nationally in total NIH funding. Candidates must hold a Ph.D., M.D., or equivalent degree and have at least three years of postdoctoral research experience. Candidates should demonstrate a strong potential to develop/maintain an externally funded research program in pharmacology or toxicology. Individuals with research expertise in drug metabolism and disposition, or translational aspects of neurodegeneration and regeneration related to Alzheimer's disease, diabetes, or traumatic brain injury are especially sought. Prospective faculty are also expected to actively participate in teaching in the graduate and professional pharmacy programs of the department. To aid faculty research, core facilities exist for proteomics, DNA microarray analyses, molecular modeling, high-throughput screening, peptide synthesis, quantitative bio-behavioral assessments and confocal/electron microscopy. The University of Kansas is especially interested in hiring faculty members who can contribute to four key campus-wide strategic initiatives: (1) Sustaining the Planet, Powering the World; (2) Promoting Well-Being, Finding Cures; (3) Building Communities, Expanding Opportunities; and (4) Harnessing Information, Multiplying Knowledge. See <http://www.provost.ku.edu/planning/themes/> for more information.

Under-represented minorities and women are encouraged to apply. To apply, please electronically submit your curriculum vitae, a three-page description of research plans and the three letters of recommendation to **Dr. Rick Dobrowsky** at dobrowsky@ku.edu. Otherwise, mail materials to **Department Pharmacology and Toxicology, 1251 Wescoe Hall Dr., University of Kansas, Lawrence, KS 66045**. Position will remain opened until filled.

The University of Kansas is an Equal Opportunity Employer.



Faculty Position Molecular and Cellular Cancer Biology Program University of Pittsburgh Cancer Institute

The University of Pittsburgh Cancer Institute (UPCI) (www.upci.upmc.edu) at the University of Pittsburgh has a strong program in molecular and cellular cancer biology (www.upci.upmc.edu/mccbp) and seeks to recruit faculty to develop outstanding research programs that bring approaches complementing our existing strengths in three broad areas. These include: genome stability; hormones and signal transduction; and mitochondria and cancer metabolism. There are opportunities for extensive collaborations within the University of Pittsburgh Medical Center and Carnegie Mellon University, as well as opportunities for participation in related graduate programs.

Specifically, we seek candidates who have an interest in the interplay between aging and cancer. We welcome applications from individuals who use state-of-the-art tools working in an array of biological systems that can translate bench science to clinical cancer applications. Candidates with a track record of independent funding and publications in high impact journals will be given the highest consideration.

Successful candidates will be expected to run a vibrant collaborative program supported by external funding. A competitive salary and research start-up package will be provided. The University Of Pittsburgh School Of Medicine is consistently among the top ten in NIH-funded medical schools in the U.S. and is located in one of America's most livable cities.

Positions will be coordinated with Departments in the University of Pittsburgh and are tenure track. To apply, please send your curriculum vitae, a one-page summary of your research plans, and three letters of recommendation to the search coordinator: **Dana Kramer, UPCI Research Pavilion, Hillman Cancer Center Suite 2.6, 5117 Centre Avenue, Pittsburgh, PA 15213-1863, email: kramerdn@upmc.edu**. Applications will be reviewed and evaluated upon receipt of full applications on an ongoing basis.

*The University of Pittsburgh is an
Affirmative Action, Equal Opportunity Employer.*



Faculty of Science
Rue Emile-Argand 11
2000 Neuchâtel
Switzerland
www.unine.ch/sciences

The University of Neuchâtel, Switzerland, invites applications for a position of

Full Professor or Assistant Professor in Geothermics

Job description: the successful candidate will establish a dynamic research program in fundamental and applied geothermics as part of a competence center for hydrogeology and geothermics. Experts in geological and geophysical exploration, characterization of aquifers and stimulated systems at intermediate/large depth, and hydrothermal/geochemical processes in such systems are particularly encouraged to apply. The successful candidate will foster synergies with local, national and international partners, contribute to teaching in the BSc and MSc curricula in English and in French after an adaptation period, and participate in administrative tasks.

Starting date: August 1st, 2013 or upon agreement.

Requirements: background in geology/Earth sciences with a PhD degree, as well as an internationally recognized research record in geothermics.

Application file: to be sent by regular mail to the Dean of the Faculty of Science, Prof. Peter Kropf, rue Emile Argand 11, 2000 Neuchâtel, Switzerland, as well as by email (one single pdf file) to doyen.sciences@unine.ch.

The applications will include a signed letter of motivation, a curriculum vitae covering the applicant's teaching and research experience, a list of research funds obtained, a list of publications and copies of academic degrees. Applicants will also provide a brief teaching statement (max. 1 page), and a description of the research projects he/she would develop at the University of Neuchâtel (max. 2 pages). The candidate will request three experts to send a signed letter of reference via email directly to the head of the Hiring Committee, Prof. D. Hunkeler daniel.hunkeler@unine.ch.

Application deadline: March 1st, 2013.

The University of Neuchâtel encourages women to apply.

Additional information: Prof. D. Hunkeler daniel.hunkeler@unine.ch or Dean of the Faculty doyen.sciences@unine.ch and www.unine.ch/sciences.



American Philosophical Society Postdoctoral Fellowship in Biological Science

Benjamin Franklin founded the American Philosophical Society (APS) in 1743 as the nation's first learned society. For many years the APS served as the nation's de facto science academy and patent office and is today the administrator of the nation's oldest scientific prize (the Magellanic Premium, first awarded in 1790).

In conjunction with its longstanding tradition of supporting the research of early career scientists and scholars in all disciplines, the APS is pleased to announce a new fellowship program: the American Philosophical Society Postdoctoral Fellowship in Biological Science. Sponsored by the Ewing Marion Kauffman Foundation, the two-year postdoctoral fellowship, to be awarded by a committee made up of eminent members of the American Philosophical Society, will support innovative research projects in fields that change each year. This first competition will fund a project in molecular biology, biochemistry, and/or the life sciences.

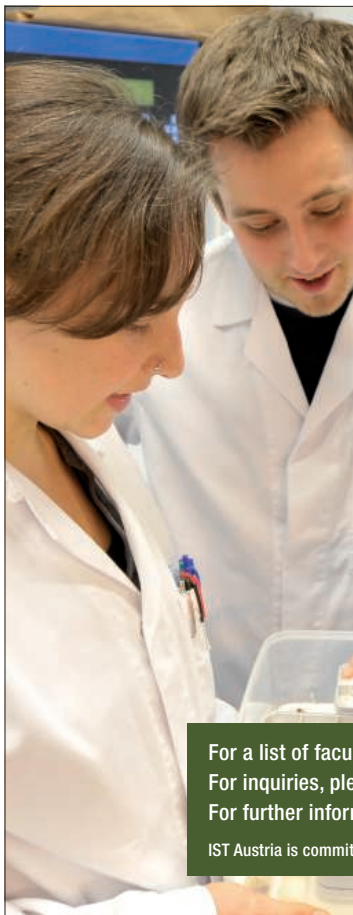
Eligibility: Applicants must have received the Ph.D. from an institution in the United States and should be either entering or already affiliated with a U.S. institution at the postdoctoral level. Applications will be judged on their innovative nature. Applications for academic year 2013-2014, eligible for renewal in academic year 2014-2015, will be based on existing problems in biochemistry and molecular biology and can include areas where either of these disciplines contribute, such as computational, evolutionary, and systems biology; anthropology; and ecology.

Award: Stipends for the fellowship are \$55,000 for the first year and \$60,000 for the second year upon approval of a renewal request.

Details and Application: Complete information on the program, the requirements, and the application materials are available at the APS website (www.amphilsoc.org/grants/biologicalscience).

Deadline and Notification: The application and the letters of support must be received by **March 1, 2013**. The committee's decision will be communicated by June 2013.

Questions may be directed to APS Director of Grants and Fellowships **Linda Musumeci**, at LMusumeci@amphilsoc.org or 215-440-3429.



ISTFELLOW

CALL FOR POSTDOCTORAL FELLOWS

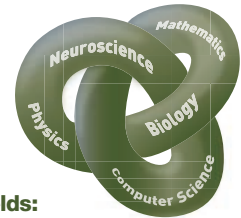
IST Austria has set up a program for exceptional postdoctoral fellows with an emphasis on interdisciplinary work. Appointments will be for 2–4 years. Applications will be accepted at any time, but fellows will be selected twice a year in April and October, with deadlines on 15th of March and September, respectively. Applicants must have the support of one or more members of the IST Austria faculty.

Benefits:

- Internationally competitive salary
- Full social security coverage
- Travel, mobility and family allowance
- Funding for conferences and scientific visits

The institute offers postdoctoral positions in the following fields:
Biology | Computer Science | Mathematics | Physics | Neuroscience

ISTFELLOW is partially funded by the European Union.



For a list of faculty members please visit www.ist.ac.at.

For inquiries, please contact istfellow@ist.ac.at.

For further information, please refer to the ISTFELLOW website: <http://ist.ac.at/istfellow>

IST Austria is committed to Equality and Diversity.



2013 Cold Spring Harbor Asia Meetings

Mechanisms and Functions of Non-apoptotic Cell Death April 15-19 www.csh.edu/asia
 Junying Yuan, Jiahui Han, Masayuki Miura, Peter Vandenabeele

Francis Crick Symposium on Neuroscience May 6-10
 Nancy Ip, Liqun Luo, Karel Svoboda

Membrane Protein Structure and Function May 13-17
 Carola Hunte, Brian Kobilka, Robert Nakamoto, Nieng Yan

Metabolism, Obesity and Obesity-associated Diseases May 20-24
 David James, Karen Reue, Baoliang Song

Vaccine Design June 3-7
 Michael Good, Yiming Shao, Ling Zhang

Plant Cell and Developmental Biology June 17-21
 Tetsuya Higashiyama, Bo Liu, Hong Ma, Zhenbiao Yang

Yersinia 11 June 24-28
 Ruitu Yang, Elisabeth Carniel, Paul Keim, Andrey Anisimov

New Advances in Optical Imaging of Live Cells and Organisms August 20-23
 Atsushi Miyawaki, Xiaoliang Sunney Xie, Rafael Yuste, Xiaowei Zhuang

Cell Signaling in Metabolism, Inflammation and Cancer September 2-6
 Michael Karin, An-Ning Lin, Zheng-Gang Liu, David Wallach

Molecular Basis of Aging and Disease September 9-13
 Adam Antebi, Jing-Dong Jackie Han, Brian Kennedy, Jan Vijn

Frontiers in Bioinformatics and Computational Biology September 23-27
 Madan Babu, Luonan Chen, Keith Dunker, Satoru Miyano, Dong Xu

Genetic, Genomic and Translational Studies of Human Leukemia October 7-11
 Scott Armstrong, Yushiaki Ito, Paul Liu, Pier-Paolo Pandolfi

CSHA/ISSCR Joint Meeting on Stem Cells in Science and Medicine October 14-18
 Zhenzhen Elefanty, Hongkui Deng, Ronald McKay, Hock-Hui Ng, Richard Young

Development, Function and Disease of Neural Circuits October 21-25
 Barry Dickson, Zhenyue He, Hitoshi Okamoto, Min Zou

Tumor Immunology and Immunotherapy October 28-November 1
 Xuetao Cao, Olivera Finn, Cornelius Melief

Nuclear Receptors and Diseases November 4-8
 Hueng-Sik Choi, David Moore, Kristina Schoonjans, Nanping Wang

Bacterial Infection and Host Defense November 18-22
 Sam Miller, Craig Roy, Feng Shao, Gisou van der Goot



For the most updated information,
 please visit our website at
www.csh-asia.org

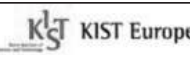


AAAS is here – bringing scientific expertise to policy making.

Good science policy is the result of politicians understanding science and scientists understanding policy. Toward this end, AAAS manages the Science & Technology Policy Fellowships program, which embeds scientists and engineers in the federal government for up to two years. From Congress to the State Department, each class of Fellows contributes to the policy-making process while getting hands-on experience at the intersection of science and policy. As a AAAS member your dues support these efforts. If you're not yet a AAAS member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/fellows





KIST Europe



**UNIVERSITÄT
DES
SAARLANDES**

The Korea Institute of Science and Technology Europe is an international research institute located on campus of the University of Saarland and is active in science and engineering projects in the field of biomedical analysis and clinical diagnostics. To strengthen our team we are searching for a

Research Group Leader for „Instrument Manufacturing and Clinical Diagnostics“

Depending on qualifications, this is combined with a **Honorary Professorship at the University of Saarland** in the department of Mechatronics.

The aim of the research work is to develop novel scientific measurement methods and instruments using nanotechnology, biotechnology or microfluidics. These methods and instruments should be of relevance to the fields of clinical diagnostics or chemical analysis. For the position, KIST Europe offers already established and soon-to-be-expanded laboratories and staff. Among the tasks of the honorary professorship is the teaching of fundamentals and application of instrument manufacturing in clinical diagnostics in the curriculum of the faculty of physics and mechatronics.

We are expecting:

- PhD in engineering or natural science
- Teaching experience as well as excellent research achievements
- Ability to raise and conduct research grant projects and to cooperate with other research institutions
- Excellent language skills in written and spoken English
- High motivation and social skills / autonomy / capacity for teamwork / own initiative

Salary level is comparable to German university (W level). Please send your written application with all usual documents to the following address until 15 January 2013:

Prof. Dr. Andreas Manz
KIST Europe Forschungsgesellschaft mbH
Campus E 71, D - 66123 Saarbrücken,
Tel. +49 (0) 681-9382 210 / manz@kist-europe.de



**UT HEALTH
SCIENCE CENTER™**
SAN ANTONIO

**Tenure Track Faculty Positions
Department of Physiology
School of Medicine**

The Department of Physiology in the School of Medicine at the University of Texas Health Science Center at San Antonio is inviting applications for two tenure-track faculty positions at the level of Assistant or Associate Professor. Prospective applicants holding a Ph.D. and/or M.D. with an outstanding record of innovative research and academic performance, and demonstrated expertise in any of the areas of systems, cellular and molecular neuroscience using cutting edge methodologies are encouraged to apply. As part of the Department's mission of expanding neuroscience research, special consideration will be accorded to candidates pursuing research in areas of neuroscience that are not currently well represented at the Health Science Center. The successful candidates will receive generous start-up packages and newly renovated laboratory space. They will be expected to establish rigorous and externally funded independent research programs, provide exemplary mentorship, and engage in productive scientific collaborations. They will become members of an Interdisciplinary Graduate Program to recruit and train graduate students. The deadline for receiving applications is **March 1, 2013**. You are encouraged to visit our website at <http://physiology.uthscsa.edu/new/> to learn about the department and the research of our current faculty. Please email a combined PDF file that includes curriculum vitae, a brief description of scientific achievements with current and future research interests (not to exceed 2 pages), and the names of three references to Physiology Search Committee [email address: physiologysearch@uthscsa.edu].

Manzoor Bhat, M.S., Ph.D.
Professor and Chairman
Zachry Foundation Distinguished Chair in Neurosciences
Department of Physiology
School of Medicine
University of Texas Health Science Center, San Antonio
7703 Floyd Curl Drive
San Antonio TX 78229

The University of Texas Health Science Center San Antonio is an Equal Employment Opportunity/Affirmative Action Employer. All faculty appointments are designated as security sensitive positions.



**UT HEALTH
SCIENCE CENTER™**
SAN ANTONIO

**Tenure Track Faculty Position
Department of Physiology
School of Medicine**

The Department of Physiology in the School of Medicine at the University of Texas Health Science Center at San Antonio is inviting applications for tenure-track faculty positions at the level of Assistant or Associate Professor. Prospective applicants holding a Ph.D. and/or M.D. with an outstanding record of innovative research and academic performance, and demonstrated expertise in genetics and/or genomics of cardiovascular development or cardiovascular pathology are encouraged to apply. As part of the Department's mission of expanding disease-related cardiovascular research, special consideration will be accorded to candidates pursuing research in areas that are not represented at the Health Science Center. The successful candidate will receive a generous start-up package, newly renovated laboratory space, and will be expected to establish rigorous and externally funded independent research programs, provide exemplary mentorship, and engage in productive scientific collaborations. The successful candidate will become a member of an Interdisciplinary Graduate Program to recruit and train graduate students. The deadline for receiving applications is **March 1, 2013**. You are encouraged to visit our website at <http://physiology.uthscsa.edu/new/> to learn about the department and the research of our current faculty. Please email a combined PDF file that includes curriculum vitae, a brief description of scientific achievements with current and future research interests (not to exceed 2 pages), and the names of three references to Physiology Search Committee [email address: physiologysearch@uthscsa.edu].

Manzoor Bhat, M.S., Ph.D.
Professor and Chairman
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School of Medicine
University of Texas Health Science Center, San Antonio
7703 Floyd Curl Drive
San Antonio TX 78229

The University of Texas Health Science Center San Antonio is an Equal Employment Opportunity/Affirmative Action Employer. All faculty appointments are designated as security sensitive positions.

Faculty Positions at ShanghaiTech University (上海科技大学)

ShanghaiTech University is a newly established research university located at Zhang-Jiang High-Tech Park in Pudong, Shanghai, China. Currently, it has four schools and several advanced research institutes: School of Physical Science and Technology, School of Life Science and Technology, School of Information Science and Technology and School of Entrepreneurship and Management with expected enrollment of 4,000 graduate and 2,000 undergraduate students. The new University is jointly supported by Chinese Academy of Sciences and Shanghai Municipal Government. The vision of ShanghaiTech is to be a globally recognized top research university for its size and profound integration of education, research and innovation by creating a dynamic people centered hub where innovative research, education, and community service meet to provide a multi-disciplinary approach to learning and to solving problems facing society. As a new university, we expect to set up new policies to support an academic environment for best practices of research, teaching and learning. The University will build state of art research and teaching facilities including large research instruments, modern library and classrooms. Our faculty will have the access to the research facilities and resources of Chinese Academy of Sciences. Shared governance will be a part of the campus culture.

We are currently seeking applicants for multiple tenure-track and tenured positions at all ranks.

Initial Research Support Package: University will provide internationally competitive start-up fund plus support of Research Associate and Post-Doctoral fellows. Laboratory space will be provided matching the research needs.

Compensation and Benefits: Salary is competitive and commensurate with experience and academic accomplishments. ShanghaiTech also offers a comprehensive benefit package including housing benefits.

1. School of Physical Science and Technology (SPST)

SPST is established to encourage interdisciplinary research particularly focused on Materials, Environment and Energy. The School is expected to have about 100 regular tenured and tenure-track faculty, 1,200 graduate and 750 undergraduate students.

Qualifications: Successful applicants should have a doctoral degree in Physical Science and Engineering as well as postdoctoral experience for junior level position. They will be expected to establish an independent, internationally recognized research program, to supervise students and to teach two courses a year. The senior position applicant is expected to be leading scientist in his/her research disciplinary. We particularly welcome those with research interests related to Energy, Materials and Environment Science and Engineering to apply.

2. School of Information Science and Technology (SIST)

SIST seeks faculty candidates in all cutting edge areas of information science and technology, with special focus on: advanced futuristic computer architecture and technology, nano-scale electronics, ultra-high speed and low power circuits, intelligent multimedia and integrated signal processing systems, next-generation computer systems, computational foundations, big data, data mining, visualization, computer vision, bio-computing, smart energy devices and systems, highly-scalable and multi-service heterogeneous networking, as well as various inter-disciplinary areas involving the foundation and applications of information science and technology.

Qualifications: Candidates must demonstrate: A strong interest in undergraduate and graduate education; Well-developed research plans and demonstrated strength; Ph.D. (Electrical Engineering, Computer Engineering, Computer Science, or closely related field); A minimum relevant research experience of 4 years.

3. School of Life Science and Technology (SLST)

SLST seeks early career scientists in these five research areas: Protein science and biotechnology; Stem cell research and regenerative medicine; Systems biology and translational medicine; Physical biology and molecular imaging; Chemical biology and innovative pharmacology.

Qualifications: The successful candidates should have an exceptional track record of research in life sciences or a closely related discipline within the last five years. Besides maintaining an active research program, the recruited candidates will also be expected to contribute to the educational missions of undergraduate and graduate programs within SLST.

Application Procedure: Submit a cover letter, a 2-3 page statement of research interests, a CV and the names and addresses of three individuals who can serve as references to the mail addresses given below:

School of Physical Science and Technology
School of Information Science and Technology
School of Life Science and Technology
School of Entrepreneurship and Management
The iHuman Institute
Shanghai Institute for Advanced Immunochemical Studies

SPST@shanghaitech.edu.cn
SIST@shanghaitech.edu.cn
SLST@shanghaitech.edu.cn
SEM@shanghaitech.edu.cn
iHuman@shanghaitech.edu.cn
SIAIS@shanghaitech.edu.cn

ShanghaiTech University, Building 2, 319 Yueyang Road, Shanghai 200031, China

Review of applications will start immediately and will continue until positions are filled.

For more information, please visit our website: www.shanghaitech.edu.cn



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/sciencecareers



**FACULTY POSITIONS
MEDICAL SCIENCES**

Washington State University Division of Health Sciences, located on the Riverpoint campus in Spokane, invites applications for two tenure-track faculty positions at the rank of Assistant or Associate Professor and one at the rank of Professor in its new Medical Sciences section. Applicants must have an earned doctorate degree in the basic, clinical, or translational medical sciences. Successful candidates will be expected to maintain an active, extramurally funded research program, to mentor graduate students and fellows, and to teach in the professional and/or graduate curricula. The successful candidate for the Professor position will additionally be expected to take a leadership role in rapidly expanding medical research in Spokane, including recruitment of faculty and programmatic development.

Areas of research interest are open, but preference will be given to candidates completing research in the areas of planned growth in our program including molecular, cellular, physiological and systems biology approaches to: neurosciences/behavioral neurosciences (sleep, addictions, pain, anesthesia), senescence and immortality (stem cells, cancer, aging, regenerative medicine), microbiology (antimicrobial resistance, virology), and metabolic diseases (obesity, diabetes, renal and cardiovascular disease). Washington State University is substantially building its research and graduate education capacity in the medical sciences. The Medical Sciences section also participates in preclinical medical education in the WWAMI program, which is a collaborative medical education program with the University of Washington School of Medicine.

Screening of applications will begin immediately and will continue until a suitable candidate is identified. To apply visit: www.wsujobs.com. Applications must include a current curriculum vitae and letter of application describing professional goals, research, and teaching experience. Before interviews commence four letters of reference will be required. **Contact Kim Noe, Administrative Manager**, at knoe@wsu.edu or 509-358-7515 for questions, assistance with the application process or confidential expressions of interest.

Women and minorities are particularly encouraged to apply. Washington State University Is An Equal Opportunity/affirmative Action Educator And Employer.



**HUMAN FRONTIER
SCIENCE PROGRAM**

**CALL FOR LETTERS OF INTENT
FOR RESEARCH GRANTS:
AWARD YEAR 2014**

HFSP supports **international** preferably **intercontinental** collaborations in **basic** life science research. Applications are invited for grants to support **innovative** and **interdisciplinary** approaches to understanding **complex mechanisms of living organisms**. Applicants are expected to develop novel lines of research distinct from their ongoing research. Preliminary results are not required.

Program Grants are for independent scientists at all stages of their careers while **Young Investigators' Grants** are for teams of scientists who are **all** within 5 years of establishing an independent laboratory and within 10 years of obtaining their PhDs. Both provide 3 years support for 2 – 4 member teams, with not more than one member from any one country, unless critical for the **innovative** nature of the project. Awards are dependent upon team size and successful teams will receive up to \$450,000 per year. The principal applicant must be located in one of the **HFSP** member countries but co-investigators may be located in any country.

For further information see the HFSP web site (www.hfsp.org). Teams must register via the web site by March 20th 2013 so as to submit a letter of intent online by the March 27th 2013 deadline.

Specific enquiries: grant@hfsp.org

Academic Positions



2011-iChEM

**Innovation Center of Chemistry
for Energy Materials (i-ChEM)**

*Xiamen University - Fudan University -
University of Science and Technology of
China*

**Faculty Appointments at
Professor/Associate Professor Level
and Postdoctoral Fellow**

The Innovation Center of Chemistry for Energy Materials (i-ChEM) is established jointly by the chemistry departments of Xiamen University, Fudan University, and University of Science and Technology of China under the "2011 Innovation Program" of the Ministries of Education and Finance of China. The mission of the Center is to establish a chemistry-based world-class center for innovative research on novel and/or strategic energy materials (including, but not limited to, synthesis, storage and conversion) at the forefront of materials chemistry as well as chemistry. The Center cordially invites applications or nominations for the following positions: Chief Scientists, Principle Investigators, Research Associates, and Postdoctoral Fellows. A number of technical and administrative personnel positions are also available.

Chief Scientist: Seven Chief Scientists positions are available for the seven divisions of the Center: (1) optimal utilization of carbon resources; (2) chemical energy storage and conversion; (3) chemistry of solar energy conversion; (4) chemical synthesis and fabrication; (5) theoretical chemistry and simulation; (6) instrumentation and methodology; (7) X-research division. The Chief Scientists are expected to be world renowned scientists in their respective fields.

Principle Investigator: To conduct cutting-edge research on respective research areas mentioned above (at the full professor level).

Research Associate: To undertake research under the leadership of a Principle Investigator (at full professor or associate professor level).

Postdoctoral Fellow: Highly motivated, young, and talented researcher who has received his/her Ph. D within the last five years.

The Center will offer successful candidates with highly competitive salaries, attractive research start-up funds, research space, and postdoctoral positions, as well as other supporting packages.

Application materials, including a cover letter; curriculum vitae; a list of names and contact details of three references; a summary of research interests and directions, should be forwarded to **Professor Yun-Bao Jiang, Head of Human Resource office of the Center** via e-mail: "2011-ichem@xmu.edu.cn". For more information please visit the website of the Center: <http://www.2011-ichem.org/>.

There's only one

DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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Smithsonian Institution

Director Tennenbaum Marine Observatories

The Smithsonian Institution seeks an innovative leader for the newly endowed Tennenbaum Marine Observatories (TMO). For more information on the TMO, please visit: <http://newsdesk.si.edu/releases/smithsonian-launches-global-marine-biodiversity-project-10-million-donation>.

The Smithsonian has launched the TMO, an initiative envisioned as a long-term, global-scale network of ecological observatories that will be dedicated to understanding changes in the structure and function of marine ecosystems. This network is committed to innovative measurements and experiments that will span traditional disciplinary boundaries and be executed in a standardized fashion over exceptional spatial and temporal scales. It is anticipated that this approach will lead to a new and fundamental understanding of our oceans that supports sustainable use.

The TMO will build upon the extraordinary strengths, capacity, and leadership of the Smithsonian Institution in marine sciences. With over 50 marine scientists, the Smithsonian expertise provides considerable depth that spans many disciplines in biology, ecology, evolutionary biology, paleobiology, anthropology, systematics, geochemistry, genetics, and other areas. The Smithsonian also has excellent infrastructure for marine science, including marine laboratories in Maryland, Florida, Belize, and Panama.

The founding director of the TMO will have the opportunity to lead a cutting-edge research program that will result in high profile, policy-relevant discoveries.

Characteristics that we seek in a director include:

- Record of experience and scholarly achievement in core areas of TMO research.
- Evidence of innovative approaches and ability to integrate ideas/concepts across traditional disciplinary boundaries.
- Clear record as an effective leader in developing and implementing a major research program and in working with diverse groups of people both inside and outside the home institution.
- Demonstrated strong organization and management skills.
- Ability to serve as the spokesperson/ambassador to other Smithsonian programs, outside collaborators, donors, and the public.
- Successful track record of competitive grant funding.

The director will be based at the National Museum of Natural History (<http://www.mnh.si.edu/>) in Washington DC, with the opportunity to establish close affiliations with one or more other research units at the Smithsonian including the Smithsonian Environmental Research Center (<http://www.serc.si.edu>), the Smithsonian Tropical Research Institute (<http://www.stri.si.edu>), and the Smithsonian Conservation Biology Institute (<http://nationalzoo.si.edu/scbi/default.cfm>). The director will guide all aspects of the design, development, implementation and growth of this new initiative, in consultation with TMO participants, and will manage all TMO activities both nationally and internationally.

The Smithsonian Institution is an Equal Opportunity Employer.

This is a full-time, permanent position located in Washington, DC with a pay range of \$150,000 to \$165,300.

This position will be open for applications starting December 20, 2012. Review of applications will start on January 21, 2013.

For further details and information on how to apply, consult <http://www.sih.si.edu/jobs.cfm> and scroll to position number EX-13-08.



Four Tenure-Track Faculty Positions in Genome-Enabled Biology

The University of New Hampshire, College of Life Sciences and Agriculture seeks to hire four new, tenure-track Assistant Professors with demonstrated interests and expertise in diverse areas of biology enabled by modern genomic analyses. We are particularly interested in building on existing strengths in: 1) behavior; 2) host/pathogen interactions; 3) nutrition; and 4) genome maintenance and evolution. Candidates must have a Ph.D. and demonstrated potential to develop and lead strong and productive research programs. They will be expected to compete successfully in national funding initiatives and to achieve national and international prominence in their fields. Individual hires will be expected to integrate their areas of research strength with existing academic programs. Successful candidates will also be expected to train graduate students and to develop and teach fundamental courses at the undergraduate and graduate levels that will contribute to academic excellence.

Complete application information is available at colsa.unh.edu/employment. Review of applications will begin on February 15, 2013 and will continue until the positions are filled.

The University actively seeks excellence through diversity among its administrators, faculty, staff and students and prohibits discrimination on the basis of race, color, religion, sex, age, national origin, sexual orientation, gender identity or expression, disability, veteran status, or marital status. Application by members of all underrepresented groups is encouraged. The University of New Hampshire is an Equal Opportunity/Equal Access/Affirmative Action institution.

All applicants will be required to apply online at <https://jobs.usnh.edu>. Please direct all inquiries to: Genome Enabled Biology Search, Lisa Buchalski, COLSA search coordinator. 603-862-3626



TEXAS A&M HEALTH SCIENCE CENTER

Faculty Positions in Biomedical Sciences Research

The Texas A&M Health Science Center **Institute of Biosciences and Technology (IBT)**, <http://ibt.tamhsc.edu/>, an internationally recognized leader in biomedical research located at the Texas Medical Center in Houston, TX, is recruiting multiple faculty members at the level of assistant and associate professor to develop research programs that complement existing strengths in Cancer Biology, Infectious Disease and Environmental Health. Candidates with interests in basic and translational research in these areas are encouraged to apply.

Applicants should have M.D., Ph.D. or M.D./Ph.D. degree in biochemistry, cellular or molecular biology or a related science and an outstanding publication record; applicants at the Assistant Professor level should have at least 3 years post-doctoral experience. The IBT is entering an expansion phase and will be recruiting multiple new faculty who will receive **highly competitive packages for salary, start-up and support for graduate education, along with outstanding laboratory and office space** in the Texas A&M Health Science Center Alkek Building in the Texas Medical Center.

Successful candidates will be expected to establish his/her independent research group, conduct highly meritorious research, establish collaborations with other investigators in the Texas Medical Center and components in the Texas A&M University System, and to obtain significant extramural funding. Applications will be received and evaluated on a rolling basis until May 31, 2013. To apply, please send a cover letter, curriculum vitae, statement of research interests, copies of two key publications, and at least three reference letters to: **Yi Xu, Ph. D., Chair of the Search Committee, Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, 2121 W. Holcombe Blvd., Houston, TX 77030-3303; E-mail: yxu@ibt.tamhsc.edu.**

The Texas A&M Health Science Center is an Affirmative Action, Equal Opportunity Employer.



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Chairs in Imaging Sciences

Salary range (non-clinical): from £68,970 p.a. Salary range (clinical): from £74,504 – £100,446 p.a.

The Clinical Sciences Centre, an MRC funded Institute based at Imperial College's Hammersmith Hospital Campus in London is seeking to increase its imaging research programmes as part of the development of a new Section of Integrative Biology and Innovative Imaging.

Our goal is to drive discovery and innovation through a fusion of clinical and basic imaging science research hosted within a vibrant multi-disciplinary Institute. Two new Groups in this section will be developing and applying imaging techniques to better understand complex biological systems and disease states in collaboration with epi/geneticists, computational scientists and cell biologists at the Institute. Our position as part of the Faculty of Medicine, Imperial College London and our co-location with Imperial College Healthcare NHS Trust will facilitate the translation of this understanding into improved diagnosis and treatment. To develop this strategy we are now seeking to appoint two outstanding clinical or non-clinical researchers at Professorial level who have the expertise and vision to contribute to the development of the Section and the overall aims of the Institute. One of the appointments will be to head the Imaging Sciences Department, and the other will be to lead a second research group within the Section. The successful candidates will have the opportunity to shape the redevelopment of the Department's infrastructure including the installation of a new clinical MR scanner and refurbished laboratories.

An open workshop for prospective candidates will be held at the Clinical Sciences Centre in London on 14 February 2013.

For further information please contact Mr Lindsay Green, CSC Head of Operations (lindsay.green@imperial.ac.uk). An information pack is available on request.

Please apply online via our website <http://www3.imperial.ac.uk/employment> (please select "Job Search", enter the job title or vacancy reference number **HM2012213** into "Keywords"). Please complete and upload an application form as directed and submit any other relevant supporting documents such as your full CV.

If you are unable to apply online, please contact Mrs Maria Monteiro, Senior Appointments Co-ordinator, e-mail: m.monteiro@imperial.ac.uk

Closing date: 6 January 2013 (Midnight GMT).

Committed to equality and valuing diversity. We are also an Athena Bronze SWAN Award winner, a Stonewall Diversity Champion and a Two Ticks Employer.



Washington University in St. Louis

SCHOOL OF MEDICINE

Faculty Positions in Biochemistry and Molecular Biophysics

The Department of Biochemistry and Molecular Biophysics at Washington University School of Medicine invites applications for tenured and tenure-track faculty positions. Successful candidates will have established a strong record of research as an independent investigator, with external funding.

Outstanding individuals working in any area of biochemistry and molecular biophysics are encouraged to apply. The candidate's research should be aimed at solving fundamental and important questions related to molecular mechanisms and addressing problems of biological or biomedical relevance. At present, research in the department spans a wide range of topics including membrane proteins, molecular motors, nucleic acid/protein interactions, protein folding and signal transduction. Additional information about the department is available at <http://www.biochem.wustl.edu>.

Applicants should email their curriculum vitae and a brief description of their research interests to the Search Committee at search@biochem.wustl.edu. Applicants should include contact information for three individuals who can write letters of recommendation. The committee will request letters as necessary.

Completed applications will be reviewed on a rolling basis, starting immediately. For full consideration, applications should be received by March 1, 2013.

Washington University is an Equal Opportunity Employer. We are committed to the recruitment of candidates traditionally underrepresented on university faculties. Individuals of any race, ethnicity, gender or sexual orientation are encouraged to apply, as are disabled individuals and veterans. The School of Medicine at Washington University is committed to finding solutions to global health problems, including ones that affect minority and disadvantaged populations.

PURDUE UNIVERSITY

Faculty Position in Evolutionary Biology Department of Biological Sciences

The Department of Biological Sciences, Purdue University, invites applicants for a tenure-track faculty position in the area of Evolutionary Biology. We expect to fill one academic-year appointment at the Assistant Professor level. We seek candidates whose research integrates the fields of ecology and evolution through investigations in subfields including behavioral ecology, community ecology, conservation biology, evolutionary genetics, phylogenetics, physiological ecology, and/or population biology. Applicants must have a Ph.D. or equivalent in an appropriate discipline such as ecology, evolution or population biology, and at least 2 years of postdoctoral experience are highly recommended. The successful applicant is expected to conduct externally funded research that addresses fundamental questions in an area listed above; teach and mentor undergraduate and graduate students in the Ecology and Evolutionary Biology curriculum; and participate in ongoing programs in the Department of Biological Sciences.

The Department has over 50 faculty members conducting research in a wide range of fields including evolutionary biology, ecology, behavior, neurobiology, microbiology/virology, structural biology, developmental biology, molecular/cell biology, and bioinformatics. Further information about the Department is available at <http://www.bio.purdue.edu>. The successful candidate will have opportunities to interact with ecologists and allied scientists across the University, including colleagues in Discovery Park's Center for the Environment and Bindley Bioscience Center. First-rate laboratory and computational facilities for analytical and systems work are available in the Department and allied Centers (e.g., Bioinformatics and Genomics Core Facilities), and field facilities are widely available in the surrounding landscape, including the Ross Biological Reserve that is owned and maintained by the Department of Biological Sciences.

Applications must be submitted electronically to <https://hiring.science.purdue.edu> as single PDF files that include detailed curriculum vitae, names and addresses of three referees, a 2 - 3 page summary of research interests, and a one-page teaching statement. Inquiries should be directed to **Evolutionary Biology Search Committee, Department of Biological Sciences, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054** or emailed to search@bio.purdue.edu. Review of applications will begin **January 7, 2013** and continue until the position is filled. A background check will be required for employment in this position.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce.

Women in Science Booklet

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Biosynthesis of alkaloids.

The post holder will contribute to a research project to deconvolute the mechanism of biosynthetic enzymes and explore the substrate specificity of these enzymes. This full time position is available from February 2013 for a period of two years, based in the laboratory in the John Innes Centre.

The post holder must have a PhD in Chemistry or a closely related discipline (or equivalent) and be able to fulfil all essential elements of the person specification. Specifically a candidate with an excellent publication record is required. Moreover, experience in biochemical projects and with molecular biology is required. Experience in chemical synthesis is desirable.

Closing date: 12 noon on 15 January 2013.

Further particulars and an application form are available on our website: www.uea.ac.uk/hr/jobs or Tel. 01603 593493.



Postdoctoral program for PhDs from Italy in Israel

The Ministry of Foreign Affairs of Italy, with CNR, ENEA and ISS, have established postdoctoral programs aimed at outstanding graduates from Italian institutions to join one of the Israeli Centers of Research Excellence operating in the following fields:

Cognitive Science Computer Algorithms
Human Disease Solar Fuels

See <http://www.itembassy.com/tel-aviv/> or <http://www.i-core.org.il/>, or also in www.cnr.it, www.enea.it, www.iss.it

Deadline for applications **January 15th, 2013**

POSITIONS OPEN

BIOCHEMISTRY FACULTY POSITION

The North Dakota State University (NDSU) Department of Chemistry and Biochemistry seeks applicants for a tenure-track **ASSISTANT PROFESSOR** position in biochemistry and/or molecular biology to begin August 2013. Exceptional candidates may be considered for a more senior appointment. Candidates must have a Ph.D. in biochemistry, molecular biology, chemistry, or a related field; evidence of strong potential to develop an internationally recognized and externally funded research program; commitment to teaching in the undergraduate and graduate curricula; and strong communication skills. NDSU is ranked as a Research University of Very High Research Activity by the Carnegie Commission on Higher Education. Information about Chemistry and Biochemistry at NDSU can be found at [website: http://www.ndsu.nodak.edu/chemistry/](http://www.ndsu.nodak.edu/chemistry/). Applications must be submitted online at [website: https://jobs.ndsu.edu/postings/2440](https://jobs.ndsu.edu/postings/2440) and should comprise a cover letter, curriculum vitae, a brief (not to exceed three pages) statement of research plans, and a one page statement of teaching goals. Applicants should arrange to have three signed letters of reference sent directly to Chair, Faculty Search Committee, e-mail: ndsu.chemistry@ndsu.edu or to: Department of Chemistry & Biochemistry, North Dakota State University, Department 2735, P.O. Box 6050, Fargo, ND 58108-6050. Review of completed applications will begin January 28, 2013 and will continue until the position is filled. *NDSU is an Equal Opportunity/Affirmative Action Employer and an NSF ADVANCE Institution. Women and traditionally under-represented groups are especially encouraged to apply. This position is exempt from North Dakota Veterans' Preference requirements.*

POSTDOCTORAL TEACHING FELLOW in Plant Ecology and Evolution

The Department of Ecology and Evolutionary Biology (EEB) at Tulane University seeks to fill the Koch-Richardson Postdoctoral Teaching Fellowship in Plant Ecology and Evolution ([website: http://tulane.edu/sse/eebio/about/kochfellow.cfm](http://tulane.edu/sse/eebio/about/kochfellow.cfm)).

The position is a two-year appointment with faculty status and a start date of July 1, 2013. The department aims to recruit an outstanding Ph.D. scientist who will merge excellence in teaching (60%), research (30%), and service (10%). Applicants are encouraged to identify a potential faculty collaborator in EEB, although those interested in botanical subjects not represented among EEB faculty will be given full consideration. Applicants should describe at least two botanical courses they would be able to teach, one at an undergraduate level, and the other at a graduate/advanced undergraduate level. An application (curriculum vitae, statement of research interests, and statement of teaching philosophy and interests), descriptions of the courses proposed to be taught, and three letters of recommendation focusing on both teaching and research excellence should be submitted electronically to the Search Committee (e-mail: ecolevol@tulane.edu). Please write "KOCH FELLOW" in the subject line. Application review will begin on February 15, 2013, and the position will remain open until filled.

Tulane University is an Equal Employment Opportunity/Affirmative Action/ADA Employer committed to excellence through diversity. All eligible candidates are invited to apply.

CLUSTER HIRING of Biomedical, Nanomaterials and Systems Faculty

The College of Engineering at Wayne State University (WSU) seeks to hire up to six tenure-track faculty in the area of biomedical, nanomaterials, and nano-systems engineering. The cluster hire is part of WSU's interdisciplinary Nano Center currently comprising more than 20 faculty members. The positions will be **ASSISTANT** or **ASSOCIATE PROFESSOR** levels for the 2013-14 academic year. Candidates should have a Ph.D. in engineering or related field. Candidates are expected to develop federally funded research programs and participate in undergraduate and graduate teaching. The full posting can be viewed at [website: http://jobs.wayne.edu](http://jobs.wayne.edu); posting #039058.

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR University of Louisville

The Institute of Molecular Cardiology (IMC), University of Louisville, has an immediate opening for a GMP Laboratory Director. This position includes a non tenure-track appointment (Assistant/Associate Professor, depending on experience and qualifications). This individual will be responsible for managing a GMP facility for human cardiac stem cells for use in clinical trials. The successful applicant will oversee expansion and phenotyping, ensure careful quality control testing, keep accurate records of all pertinent aspects of cell cultures, write, and implement Standard Operating Procedures (SOPs), and comply with all federal reporting. Requirements include a Ph.D./M.D./DVM or equivalent, and a minimum two to three years of GMP laboratory experience. Applicants with expertise in stem cell biology will be strongly preferred. The IMC is home to several established research programs in diverse areas of cardiovascular pathophysiology and stem cell biology. We offer a competitive salary and startup support, a highly collegial cardiovascular environment, and abundant opportunities. Submit a cover letter, curriculum vitae, and three references to: **Roberto Bolli, M.D., Director, Institute of Molecular Cardiology; ACB 3rd Floor, 550 South Jackson Street, Louisville, Kentucky 40292. E-mail: rbolli@louisville.edu.**

University of Louisville is an Equal Opportunity/Affirmative Action Employer; women and minorities are encouraged to apply.

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